Reference 5

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C12N 15/62, C07K 15/00, C12P 21/00

A2

(11) International Publication Number: WO 94/16085

(43) International Publication Date: 21 July 1994 (21.07.94)

(21) International Application Number: PCT/US93/12687

(22) International Filing Date: 30 December 1993 (30.12.93)

(30) Priority Data:
07/998,271 30 December 1992 (30.12.92) US

(71) Applicant: ZYMOGENETICS, INC. [US/US]; 4225 Roosevelt Way NE, Seattle, WA 98105 (US).

(72) Inventor: IRANI, Meher, H.; 3503 NE 100th, Seattle, WA 98125 (US).

(74) Agent: PARKER, Gary, E.; ZymoGenetics, Inc., 4225 Roosevelt Way NE, Seattle, WA 98105 (US).

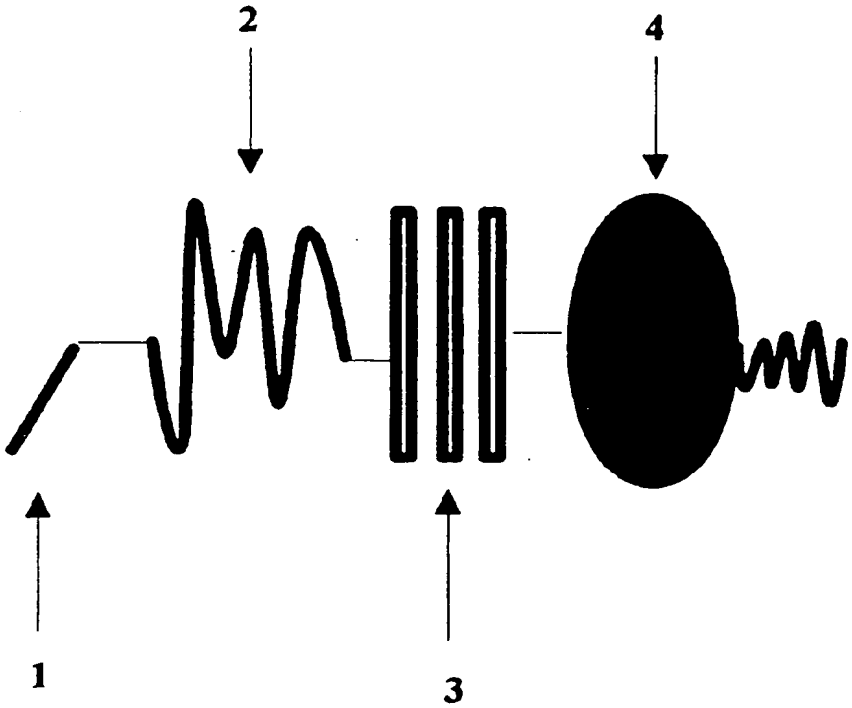
(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: HYBRID PROTEINS HAVING CROSS-LINKING AND TISSUE-BINDING ACTIVITIES

(57) Abstract

Hybrid proteins having cross-linking and tissue-binding activities, DNA molecules encoding such proteins and methods for producing the hybrid proteins from recombinant host cells are disclosed. The hybrid proteins disclosed herein are useful in tissue sealant and wound healing formulations.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

Description

Hybrid Proteins Having Cross-Linking and Tissue-Binding Activities

Technical Field

The present invention relates generally toward methods for producing recombinant hybrid proteins, and more specifically, to methods for producing hybrid proteins from host cells through the use of recombinant DNA techniques.

Background of the Invention

The utilization of tissue sealants to replace or augment the use of mechanical wound closure devices has expanded in recent years in many surgical and trauma applications. Tissue sealants include biological adhesives (e.g. fibrin-based adhesives) and synthetic preparations (e.g. cyanoacrylates). It is widely acknowledged that the use of synthetic preparations of tissue sealants is limited due to their toxicity and limited applications. Biological tissue adhesives have demonstrated utility in cases where the use of mechanical devices to close wounds is insufficient, such as in joining blood vessels, closing holes in the dura, and in surgery on small or delicate tissues such as in the eye or ear.

Fibrin-based biological tissue adhesives generally contain fibrinogen, factor XIII and thrombin as principal ingredients, although in practice biological tissue adhesives are derived from whole blood and contain additional blood proteins. The fibrinogen and factor XIII components of these adhesives are prepared from pooled human plasma by cryoprecipitation (e.g. U.S. Patents No. 4,377,572; 4,362,567; 4,909,251), by ethanol precipitation (e.g. U.S. Patent No. 4,442,655) or from single donor plasma (e.g. U.S. Patent No. 4,627,879; Spotnitz et al., Am. Surg. 55: 166-168, 1989). The resultant

fibrinogen/factor XIII preparation is mixed with bovine thrombin immediately before use to convert the fibrinogen to fibrin and activate the factor XIII, thus initiating coagulation of the adhesive.

Fibrin-based tissue adhesives, in their current form, have significant drawbacks that include poor standardization, lack of quality control from batch to batch and the possibility of transmission of human immunodeficiency virus (HIV), hepatitis virus and other etiologic agents. While recombinant production of thrombin and factor XIII have been reported, and while these proteins might be used in biological tissue adhesives, the biological tissue adhesives still rely on large amounts of fibrinogen that is obtained from pooled human blood. At present, current fibrin(ogen)-based tissue adhesives are not approved for use in the United States.

There is therefore a need in the art for tissue adhesive components, particularly components that facilitate cross-linking to improve clot strength, that are prepared at high levels with reproducible activity levels and which do not carry the possibility of transmission of viral or other etiologic agents. The present invention addresses these needs by providing recombinant hybrid proteins that provide cross-linking and tissue-adhesive properties and that may be prepared at high levels.

Disclosure of the Invention

Briefly stated, the present invention provides hybrid proteins having cross-linking and tissue-binding activities, DNA molecules encoding such hybrid proteins and methods for producing hybrid proteins by recombinant means. In one aspect, In one aspect of the invention, the hybrid proteins comprise a tissue-binding domain from a first protein covalently linked to a cross-linking domain from a second protein. Within a related aspect of the

invention, the tissue-binding domain of the first protein is a heparin binding domain of thrombospondin, a heparin binding domain of fibronectin, a collagen binding domain of fibronectin or a cell binding domain of fibronectin. Within a preferred embodiment, the tissue-binding domain of the first protein comprises the amino acid sequence of Sequence ID No. 6 from Alanine, amino acid 2 to Glutamic acid, amino acid number 926. Within another related aspect of the invention, the cross-linking domain of the second protein comprises the carboxy-terminal 103 amino acids of loricrin, the ten amino acid repeat beginning with glutamine amino acid number 496 of involucrin or the 400 amino-terminal amino acids of the fibrinogen α chain. Within a preferred embodiment of the invention, the tissue-binding domain of the second protein comprises the amino acid sequence of Sequence ID No. 6 from Glycine, amino acid number 928 to Proline, amino acid number 1336. Within a particularly preferred embodiment, the hybrid protein comprises the amino acid sequence of Sequence ID No. 6 from alanine, amino acid number 2 to proline, amino acid number 1336.

The present invention provides DNA molecules encoding hybrid proteins of the present invention comprising a first DNA segment encoding a tissue-binding domain from a first protein joined to a second DNA segment encoding a cross-linking domain from a second protein. In one embodiment, the first DNA segment comprises the nucleotide sequence of Sequence ID No. 5 from nucleotide 3 to nucleotide 2780. In another embodiment, the second DNA segment comprises the nucleotide sequence of Sequence ID No. 5 from nucleotide 2784 to nucleotide 4013. In a preferred embodiment, the DNA molecule comprises the nucleotide sequence of Sequence ID Number 5 from nucleotide 3 to nucleotide 4013.

In related embodiments of the invention, DNA constructs are provided which comprise a DNA molecule encoding a hybrid protein, whereins said DNA molecule

comprises a first DNA segment encoding a tissue-binding domain from a first protein joined to a second DNA segment encoding a cross-linking domain from a second protein and wherein said DNA molecule is operably linked to other DNA segments required for the expression of the DNA molecule. Other embodiments of the invention concern host cells containing the DNA constructs of the present invention and methods of producing hybrid proteins.

Brief Description of the Drawings

Figure 1 discloses a representative hybrid protein containing (1) an N-terminal end-to-end inter-chain cross-linking domain, (2) a domain that promotes inter-chain cross-linking; (3) a domain that confers tissue binding activity; and (4) a carboxy-terminal domain that promotes end-to-end inter-chain cross-linking.

Figures 2-5 disclose absorbance time courses of representative cross-linking assays carried out in the presence of varying levels of factor XIII (activated to factor XIIIa via thrombin during the assay) or factor XIIIa.

Detailed Description of the Invention

The present invention provides novel hybrid proteins having cross-linking and tissue adhesive activities. The hybrid proteins comprise a cross-linking domain from a first protein covalently linked to a tissue-binding domain from a second protein. The hybrid proteins of the present invention are capable of cross-linking to themselves and to other proteins such as fibrin and fibrinogen and are capable of adhering to cell surfaces and/or extracellular matrix components. While not wishing to be bound by a graphical representation, Figure 1 shows a representative hybrid protein containing an N-terminal end-to-end inter-chain cross-linking domain; a domain that promotes inter-chain cross-linking; a domain that confers tissue binding activity; and a carboxy-terminal domain

that promotes end-to-end inter-chain cross-linking. As used herein, cross-linking refers to the formation of covalent bonds between polypeptides.

The hybrid proteins of the present invention are useful as components of tissue sealant formulations to provide matrix material and to improve clot strength over a wound site, and as components in formulations that promote wound healing. The proteins of the present invention may contain native (i.e. wild-type) protein domains as well as domains that are allelic variants and genetically engineered or synthetic variants of the respective naturally occurring domains. Such variants are characterized by the presence of conservative amino acid substitutions and/or other minor additions, substitutions or deletions of amino acids.

As used within the context of the present invention, tissue-binding domains include protein domains containing amino acid sequences that facilitate adherence to cell surfaces and/or to extracellular matrix components such as collagen, fibronectin, hyaluronic acid and glycosaminoglycans. Fibronectin, for example, contains the sequence Gly-Arg-Gly-Asp-Ser (from amino acid 1614 through amino acid 1618 of Sequence I.D. No. 3) that has been shown to be central to cell recognition by the fibronectin receptor (for review see Yamada, Current Opinion in Cell Biology 1: 956-963, 1989). The heparin binding domains of fibronectin (Sekiguchi et al., Proc. Natl. Acad. Sci. USA 77: 2661-2665, 1980), and thrombospondin (Zardi et al., EMBO J. 6: 2337-3342, 1987 and Gutman and Kornblihtt, Proc. Natl. Acad. Sci. USA 84: 7179-7182, 1987) contain sequences that recognize heparin sulfate-containing glycosaminoglycans which are extracellular matrix components. The collagen binding domain of fibronectin (Sekiguchi et al. ibid., 1980) contains amino acid sequences that bind to the extracellular matrix component collagen.

Particularly preferred tissue-binding domains are the heparin binding domain of fibronectin, comprising the sequence of amino acids of Sequence I.D. No. 2 from alanine, amino acid number 1812 to valine, amino acid number 2171; the collagen binding domain of fibronectin, comprising the sequence of amino acids of Sequence I.D. No. 2 from glycine, amino acid number 282 to serine, amino acid number 608; and the amino terminal 229 amino acids of thrombospondin. In this regard, a particularly preferred tissue-binding domain is the cell-binding domain of fibronectin, comprising the sequence of amino acids of Sequence I.D. No. 3 from alanine, amino acid number 1357 to glutamic acid, amino acid number 1903. It will be evident to one skilled in the art that smaller portions of the cell-binding domain of fibronectin may be used within the hybrid proteins of the present invention, more particularly the sequence of amino acids of Sequence I.D. No. 3 from isoleucine, number 1532 through threonine, amino acid number 1631. As noted above, it is generally accepted that the sequence Gly-Arg-Gly-Asp-Ser (Amino acids 1614 to 1618 of Sequence I.D. No. 3) is central to cell recognition by fibronectin.

Cross-linking domains suitable for use in the hybrid proteins of the present invention are protein domains which contain amino acid sequences required for the formation of specific covalent bonds between peptide chains. In a preferred embodiment the inter-chain cross-links are covalent bonds formed by the action of a transglutaminase such as factor XIII, tissue transglutaminase, prostate transglutaminase, keratinocyte transglutaminase, epidermal transglutaminase or placental transglutaminase. Transglutaminases catalyze the formation of ϵ-(γ-glutamyl)lysine bonds between specific glutamine and lysine residues. However, other inter-chain cross-links, such as those formed by disulfide bonds, are also suitable cross-links. Suitable cross-linking domains include domains from the fibrinogen α chain, the

glutamine/lysine rich domains of loricrin that are involved in isodipeptide cross-link formation (Hohl et al., J. Biol. Chem. 266: 6626-6636, 1991), and at least one of the 10 amino acid-long repeats of involucrin (Cell 46: 583-589, 1986 and Etoh et al., Biochem. Biophys. Res. Comm. 136: 51-56, 1986). Preferred cross-linking domains are the carboxy-terminal 103 amino acids of loricrin (Hohl et al., ibid.) and the ten-amino acid repeat beginning with glutamine, amino acid number 496 of involucrin (Simon et al. (J. Biol. Chem. 263: 18093-18098, 1988). A particularly preferred cross-linking domain comprises the 400 amino-terminal amino acids of the fibrinogen α chain (Doolittle et al., Nature 280: 464-468, 1979; Rixon et al., Biochemistry 22: 3250-3256, 1983). More particularly, the amino acid sequence of Sequence ID No. 6 from Glycine, amino acid number 928 to Proline, amino acid number 1336 is preferred.

Although the hybrid proteins of the present invention may consist essentially of covalently linked cross-linking and tissue binding domains, they may further contain domains that facilitate end-to-end covalent cross-linking. The γ chain of fibrinogen contains a domain that facilitates end-to-end cross-linking to another γ chain via ε-(γ-glutamyl)lysine bonds. This domain includes at least the 19 carboxy-terminal amino acids and more preferably includes the amino-terminal 275 amino acids of the fibrinogen γ chain. The α chain of fibrinogen contains an amino-terminal domain that is involved in interchain disulfide bond formation between α chains. This domain includes the amino-terminal portion of the α chain of fibrinogen from glycine, amino acid 36 to glycine, amino acid 67 of Sequence ID Number 4.

As will be evident to one skilled in the art, the hybrid proteins of the present invention may contain domains of human and other animal proteins. Proteins containing domains suitable for use in the present invention from human and other animals and the DNA

molecules encoding such proteins have been reported. Involucrin, loricrin, fibrinogen and fibronectin, for example, have been studied in a variety of animals. DNA sequences encoding primate, canine and porcine involucrin have been reported (Djian and Green, Mol. Biol. Evol. 9: 417-432, 1992; Djian and Green, Proc. Natl. Acad. Sci. USA 88: 5321-5325, 1991 and Tseng and Green, Mol. Biol. Evol. 7: 293-302, 1990). Mehrel et al. (Cell 61: 1103-1112, 1990) have reported a DNA sequence encoding mouse loricrin. DNA sequences encoding rat and frog fibrinogen gamma chain have been reported (Haidaris and Courtney, Blood 79: 1218-1224, 1992 and Bhattacharya et al., Mol. Cell. Endocrinol. 72: 213-220, 1990; respectively). DNA sequences encoding chicken and lamprey fibrinogen α chains have been reported by Weissbach and Greininger (Proc. Natl. Acad. Sci. USA 87: 5198-5202, 1990) and Pan and Doolittle (Proc. Natl. Acad. Sci. USA 89: 2066-2070, 1992), respectively. DNA sequences encoding bovine and rat fibronectin have been reported by Petersen et al. (Proc. Natl. Acad. Sci. USA 80: 137-141, 1983) and Schwarzbauer et al., (Cell 35: 421-431, 1983). In general, it is preferred to prepare proteins that contain component domains from a single species to minimize the possibility of immunogenicity. Thus, the present invention provides hybrid proteins that can be used in human and veterinary medicine.

According to the present invention hybrid proteins having cross-linking and tissue adhesive activities are produced recombinantly from host cells transformed with a DNA construct comprising a DNA segment encoding a cross-linking domain from a first protein joined to a DNA segment encoding a tissue-binding domain from a second protein. As used within the context of the present invention, two or more DNA coding sequences are said to be joined when, as a result of in-frame fusions between the DNA coding sequences or as a result of the removal of intervening sequences by normal cellular

processing, the DNA coding sequences can be translated into a polypeptide fusion. Unless otherwise noted, the DNA segments may be joined in any order to result in a DNA coding sequence that can be translated into a polypeptide chain. Thus, the DNA segment encoding the tissue-binding domain may be joined to the 5′ or the 3′ end of the DNA segment encoding the cross-linking domain. However, as will be evident to one skilled in the art, the production of hybrid proteins that additionally include domains that facilitate end-to-end cross-linking will require that the DNA segments encoding such domains be positioned at the 5′ and 3′ termini of the molecules.

Thus the present invention also provides isolated DNA molecules encoding hybrid proteins comprising a cross-linking domain from a first protein covalently linked to a tissue-binding domain from a second protein. In general, cDNA sequences are preferred for carrying out the present invention due to their lack of intervening sequences which can lead to aberrant RNA processing and reduced expression levels. DNA molecules encoding human fibronectin (Dufour et al., Exper. Cell Res. 193: 331-338, 1991) and a human fibrinogen α chain (Rixon et al., Biochemistry 22: 3250-3256, 1983) may be obtained from libraries prepared from liver cells according to standard laboratory procedures. It will be understood however, that suitable DNA sequences can also be obtained from genomic clones or can be synthesized de novo according to conventional procedures. If partial clones are obtained, it is necessary to join them in proper reading frame to produce a full length clone, using such techniques as endonuclease cleavage, ligation, and loop-out mutagenesis.

DNA sequences encoding hybrid proteins of the present invention may be prepared from cloned DNAs using conventional procedures of endonuclease cleavage, exonuclease digestion, ligation and in vitro mutagenesis. Alternatively, DNA sequences encoding the cross-linking

and tissue-binding domains, such as those mentioned above, may be synthesized using standard laboratory techniques.

An exemplary DNA molecule encoding a hybrid protein having cross-linking and tissue-binding activities may be prepared by joining a DNA segment encoding at least the cell-binding domain of fibronectin and a DNA segment encoding at least an inter-chain cross-linking domain of fibrinogen at a convenient restriction site using synthetic adapters to facilitate in-frame joining of the DNA segments. Alternatively, such DNA segments encoding hybrid proteins of the present invention may be prepared by joining the two domains at a convenient restriction site followed by loop-out mutagenesis to precisely remove unnecessary sequences and directly join the DNA segment encoding the cell-binding domain of fibronectin with the DNA segment encoding the cross-linking domain of fibrinogen.

DNA segments encoding the hybrid proteins of the instant invention are inserted into DNA constructs. As used within the context of the present invention, a DNA construct is understood to refer to a DNA molecule, or a clone of such a molecule, either single- or double-stranded, which has been modified through human intervention to contain segments of DNA combined and juxtaposed in a manner that would not otherwise exist in nature. DNA constructs of the present invention comprise a first DNA segment encoding a hybrid protein operably linked to additional DNA segments required for the expression of the first DNA segment. Within the context of the present invention, additional DNA segments will generally include promoters and transcription terminators, and may further include enhancers and other elements.

DNA constructs may also contain DNA segments necessary to direct the secretion of a polypeptide or protein of interest. Such DNA segments may include at least one secretory signal sequence. Secretory signal sequences, also called leader sequences, prepro sequences

and/or pre sequences, are amino acid sequences that act to direct the secretion of mature polypeptides or proteins from a cell. Such sequences are characterized by a core of hydrophobic amino acids and are typically (but not exclusively) found at the amino termini of newly synthesized proteins. DNA segments encoding secretory signal sequences are placed in-frame and in the correct spatial relationship to the DNA segment encoding the protein of interest in order to direct the secretion of the protein. Very often the secretory peptide is cleaved from the mature protein during secretion. Such secretory peptides contain processing sites that allow cleavage of the secretory peptides from the mature proteins as they pass through the secretory pathway. A preferred processing site is a dibasic cleavage site, such as that recognized by the Saccharomyces cerevisiae KEX2 gene. A particularly preferred processing site is a Lys-Arg processing site. Processing sites may be encoded within the secretory peptide or may be added to the peptide by, for example, in vitro mutagenesis.

Preferred secretory signals include the α factor signal sequence (pre-pro sequence: Kurjan and Herskowitz, Cell 30: 933-943, 1982; Kurjan et al., U.S. Patent No. 4,546,082; Brake, U.S. Patent No. 4,870,008), the PHO5 signal sequence (Beck et al., WO 86/00637), the BAR1 secretory signal sequence (MacKay et al., U.S. Patent No. 4,613,572; MacKay, WO 87/002670), the SUC2 signal sequence (Carlsen et al., Molecular and Cellular Biology 3: 439-447, 1983). Alternately, a secretory signal sequence may be synthesized according to the rules established, for example, by von Heinje (European Journal of Biochemistry 133: 17-21, 1983; Journal of Molecular Biology 184: 99-105, 1985; Nucleic Acids Research 14: 4683-4690, 1986).

Secretory signal sequences may be used singly or may be combined. For example, a DNA segment encoding a first secretory signal sequence may be used in combination with a DNA segment encoding the third domain of barrier

(described in U.S. Patent No. 5,037,243, which is incorporated by reference herein in its entirety). The DNA segment encoding the third domain of barrier may be positioned in proper reading frame 3' of the DNA segment of interest or 5' to the DNA segment and in proper reading frame with both the DNA segment encoding the secretory signal sequence and the DNA segment of interest.

The choice of suitable promoters, terminators and secretory signals is well within the level of ordinary skill in the art. Methods for expressing cloned genes in Saccharomyces cerevisiae are generally known in the art (see, "Gene Expression Technology," Methods in Enzymology, Vol. 185, Goeddel (ed.), Academic Press, San Diego, CA, 1990 and "Guide to Yeast Genetics and Molecular Biology," Methods in Enzymology, Guthrie and Fink (eds.), Academic Press, San Diego, CA, 1991; which are incorporated herein by reference). Transformation systems for other yeasts, including Hansenula polymorpha, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces fragilis, Ustilago maydis, Pichia pastoris, Pichia guillermondil and Candida maltosa are known in the art. See, for example, Gleeson et al., J. Gen. Microbiol. 132:3459-3465, 1986 and Cregg, U.S. Patent No. 4,882,279.

Proteins of the present invention can also be expressed in filamentous fungi, for example, strains of the fungi Aspergillus (McKnight et al., U.S. Patent No. 4,935,349, which is incorporated herein by reference). Methods for transforming Acremonium chrysogenum are disclosed by Sumino et al., U.S. Patent No. 5,162,228, which is incorporated herein by reference.

Other higher eukaryotic cells may also be used as hosts, including insect cells, plant cells and avian cells. Transformation of insect cells and production of foreign proteins therein is disclosed by Guarino et al., U.S. Patent No. 5,162,222 and Bang et al., U.S. Patent No. 4,775,624, which are incorporated herein by reference. The use of Agrobacterium rhizogenes as a vector for

expressing genes in plant cells has been reviewed by Sinkar et al., J. Biosci. (Bangalore) 11:47-58, 1987.

Expression of cloned genes in cultured mammalian cells and in E. coli, for example, is discussed in detail in Sambrook et al. (Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY, 1989; which is incorporated herein by reference). In addition to E. coli, Bacillus and other genera are useful prokaryotic hosts for expressing foreign proteins. As would be evident to one skilled in the art, one could express the proteins of the instant invention in other host cells such as avian, insect and plant cells using regulatory sequences, vectors and methods well established in the literature.

In yeast, suitable vectors for use in the present invention include YRp7 (Struhl et al., Proc. Natl. Acad. Sci. USA 76: 1035-1039, 1978), YEp13 (Broach et al., Gene 8: 121-133, 1979), POT vectors (Kawasaki et al, U.S. Patent No. 4,931,373, which is incorporated by reference herein), pJDB249 and pJDB219 (Beggs, Nature 275:104-108, 1978) and derivatives thereof. Preferred promoters for use in yeast include promoters from yeast glycolytic genes (Hitzeman et al., J. Biol. Chem. 255: 12073-12080, 1980; Alber and Kawasaki, J. Mol. Appl. Genet. 1: 419-434, 1982; Kawasaki, U.S. Patent No. 4,599,311) or alcohol dehydrogenase genes (Young et al., in Genetic Engineering of Microorganisms for Chemicals, Hollaender et al., (eds.), p. 355, Plenum, New York, 1982; Ammerer, Meth. Enzymol. 101: 192-201, 1983). In this regard, particularly preferred promoters are the TPI1 promoter (Kawasaki, U.S. Patent No. 4,599,311, 1986) and the ADH2-4^{C} promoter (Russell et al., Nature 304: 652-654, 1983; Irani and Kilgore, U.S. Patent Application Serial No. 07/631,763, CA 1,304,020 and EP 284 044, which are incorporated herein by reference). The expression units may also include a transcriptional terminator. A

preferred transcriptional terminator is the TPI1 terminator (Alber and Kawasaki, ibid.).

Host cells containing DNA constructs of the present invention are then cultured to produce the hybrid proteins. The cells are cultured according to standard methods in a culture medium containing nutrients required for growth of the particular host cells. A variety of suitable media are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins, minerals and growth factors. The growth medium will generally select for cells containing the DNA construct by, for example, drug selection or deficiency in an essential nutrient which is complemented by a selectable marker on the DNA construct or co-transfected with the DNA construct.

Selection of a medium appropriate for the particular host cell used is within the level of ordinary skill in the art. Yeast cells, for example, are preferably cultured in a chemically defined medium, comprising a non-amino acid nitrogen source, inorganic salts, vitamins and essential amino acid supplements. The pH of the medium is preferably maintained at a pH greater than 2 and less than 8, preferably at pH 6.5. Methods for maintaining a stable pH include buffering and constant pH control, preferably through the addition of sodium hydroxide or ammonium hydroxide. Preferred buffering agents include succinic acid and Bis-Tris (Sigma Chemical Co., St. Louis, MO). Yeast cells having a defect in a gene required for asparagine-linked glycosylation are preferably grown in a medium containing an osmotic stabilizer. A preferred osmotic stabilizer is sorbitol supplemented into the medium at a concentration between 0.1 M and 1.5 M, preferably at 0.5 M or 1.0 M. Cultured mammalian cells are generally cultured in commercially available serum-containing or serum-free media.

The recombinant hybrid proteins expressed using the methods described herein are isolated and purified by

conventional procedures, including separating the cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, e.g. ammonium sulfate, purification by a variety of chromatographic procedures, e.g. ion exchange chromatography or affinity chromatography, or the like. Methods of protein purification are known in the art (see generally, Scopes, R., Protein Purification, Springer-Verlag, NY (1982), which is incorporated herein by reference) and may be applied to the purification of the recombinant proteins of the present invention.

The hybrid proteins of the present invention may be used as components of tissue adhesives. It is preferred that the tissue adhesives be formulated to provide a concentration of the hybrid proteins of the present invention of between about 5 mg/ml to 100 mg/ml, with concentrations in the range of 35 to 50 mg/ml being particularly preferred. As disclosed above, tissue adhesives generally contain factor XIII and thrombin. Additional components may also be included in the tissue adhesive formulations. These additional components include growth factors such as PDGF, bFGF, TGFa, or EGF and protease inhibitors, such as aprotinin, transexamic acid, alpha-2 plasmin inhibitor, alpha-1-antitrypsin or the Pittsburgh mutant of alpha-1-antitrypsin (Arg-358 alpha-1-antitrypsin). The tissue adhesives may also contain salts, buffering agents, reducing agents, bulking agents, and solubility enhancers. Albumin, NaCl, $CaCl_2$, citrate and phosphate buffers, for example, may be included. Preferably, the tissue adhesives of the present invention are prepared as lyophilized powders, liquid concentrates of ready-to-use liquids. Lyophilized powders are preferred for ease of handling and storage.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1 -Subcloning and Modification of ADH2 Promoters

An ADH2-4^{C} promoter was constructed as described in co-pending U.S. Patent Application 07/631,763, CA 1,304,020 and EP 284 044, which are incorporated herein by reference. A DNA construct comprising the complete ADH2-4^{C} promoter mutagenized at the 3′ end to place an Eco RI site in place of the translation start codon, designated p410-4^{C} (deposited with the American Type Culture Collection (12301 Parklawn Dr., Rockville, MD 20852) under accession number 68861) was used as the source of the ADH2-4^{C} promoter.

A PAP-I cDNA (U.S. Patent No. 4,937,324) was joined with the ADH2-4^{C} promoter. Plasmid pAP1.7, comprising the 1.7 kb cDNA in pUC18, was cut with Nco I and Bam HI, and the linearized plasmid was isolated through two rounds of gel purification. The ADH2-4^{C} promoter from p410-4^{C} was joined to the 5′ end of the PAP-I cDNA via an Eco RI-Nco I adapter. The 1.2 kb Bam HI-Eco RI promoter fragment from p410-4^{C}, Eco RI-Nco I adapter and the Nco I-Bam HI linearized pAP1.7 plasmid were ligated. The resultant plasmid was designed pPR1. The presence of the correct promoter fusion was confirmed by DNA sequencing.

A yeast expression vector comprising the ADH2-4^{C} promoter, the PAP-I cDNA and the TPI1 terminator was constructed. Plasmid pZUC13 (comprising the S. cerevisiae chromosomal LEU2 gene and the origin of replication from S. cerevisiae 2 micron plasmid inserted into pUC13 and constructed in a manner analogous to pZUC12, described in published EP 195,691, using the plasmid pMT212, which is described in published EP 163 529) was cut with Bam HI. Plasmid pPR1 was digested completely digested with Bam HI and partially digested with Sac I to isolate the 2.1 kb ADH2-4^{C} promoter-PAP-I cDNA fragment. Plasmid pTT1 (described in detail below) was digested with Sac I and

Bam HI to isolate the 0.69 bp TPI1 terminator fragment. The Bam HI-Sac I fragment from pPR1 and the Sac I-Bam HI fragment from pTT1 were ligated with the Bam HI-linearized pZUC13. A plasmid containing the expression unit was designated pZ3.

Example 2 - Subcloning of the TPI1 terminator

The yeast TPI1 terminator fragment was obtained from plasmid p270 described by Murray and Kelly (U.S. Patent 4,766,073, which is incorporated by reference herein in its entirety). Plasmid p270 contains the TPI1 terminator inserted as and Xba I-Bam HI fragment into YEp13. Alternatively, the TPI1 terminator may be obtained from plasmid pM220 (deposited with American Type Culture Collection as an E. coli RR1 transformant under accession number 39853) by digesting the plasmid with Xba I, and Bam HI and purifying the TPI1 terminator fragment (~700 bp).

The TPI1 terminator was removed from plasmid p270 as a Xba I-Bam HI fragment. This fragment was cloned into pUC19 along with another fragment containing the TPI1 promoter fused to the CAT (chloramphenicol acetyl transferase) gene to obtain a TPI1 terminator fragment with an Eco RV end. The resultant plasmid was designated pCAT. The TPI1 terminator was then cut from pCAT as an Eco RV-Bam HI fragment and cloned into pIC19H (Marsh et al., Gene 32:481-486, 1984) which had been cut with the same enzymes, to obtain pTT1 (disclosed in U.S. Patent No. 4,937,324, which is incorporated herein by reference).

Example 3 - Construction of Yeast Vectors pDPOT and pRPOT

Plasmid pDPOT was derived from plasmid pCPOT (ATCC No. 39685) by replacing the 750 bp Sph I-Bam HI fragment of pCPOT containing 2 micron and pBR322 sequences with a 186 bp Sph I-Bam HI fragment derived from the pBR322 tetracycline resistance gene.

Plasmid pRPOT was derived from plasmid pDPOT by replacing the Sph I-Bam HI fragment with a polylinker. Plasmid pDPOT was digested with Sph I and Bam HI to isolate the 10.8 kb fragment. Oligonucleotides ZC1551 and ZC1552 (Sequence ID Nos. 7 and 8) were designed to form an adapter with a Bam HI adhesive end and an Sph I adhesive end flanking Sma I, Sst I and Xho I restriction sites. Oligonucleotides ZC1551 and ZC1552 (Sequence ID Nos. 7 and 8) were kinased and annealed to form the Bam HI-Sph I adapter. The 10.8 kb pDPOT fragment was circularized by ligation with the ZC1551/ZC1552 adapter (Sequence ID Nos. 7 and 8). The resultant plasmid was termed pRPOT.

Example 4 - Construction of a Fibrinogen:Fibronectin Hybrid cDNA Expression Vector

A. Construction of pFN14A

A DNA construct containing a DNA segment encoding the fibronectin cell-binding domain operably linked to the ADH2-4^{c} promoter in plasmid pUC19 was constructed. The fibronectin coding sequence was obtained from plasmid pFH103 (Dufour et al., Exper. Cell Res. 193: 331-338, 1991). Plasmid pFH103 was digested with Nco I and Xba I to isolate the 4 kb fragment containing the fibronectin coding sequence. Oligonucleotides ZC2052 and ZC2053 (Sequence ID Nos. 9 and 10) were designed to provide, upon annealing, an adapter containing a 5′ Eco RI adhesive end, an internal Nco I site, a DNA segment encoding a methionine and amino acids 979 through 981 of Sequence ID Number 2 and a 3′ Nco I adhesive end that destroys the Nco I site. Oligonucleotides ZC2052 and ZC2053 (Sequence ID Nos. 9 and 10) were annealed and ligated with the 4 kb Nco I-Xba I fibronectin fragment into Eco RI-Xba I linearized pUC19. The resultant plasmid was designated pFN4.

Plasmid pFN4 was digested with Hind III and Apa I to isolate the 3.3 kb fibronectin fragment. Oligonucleotides ZC2493 and ZC2491 (Sequence ID Nos. 12

and 11) were designed to provide, when annealed, an Apa I-Xba I adapter encoding the amino acids Pro and Phe followed by a stop codon. The oligonucleotides were annealed and combined with the 3.3 kb Hind III-Apa I fragment and Hind III-Xba I linearized pUC19 to form plasmid pFN7. Plasmid pFN7 comprises a DNA segment encoding amino acids 1273-2186 of Sequence ID Number 2 followed by an in-frame stop codon.

The ADH2-4^{c} promoter was joined to the 5′ end of the fibronectin cDNA in plasmid pFN5. Plasmid pFN4 was digested with Nco I and Hind III to isolate the 0.89 kb fibronectin coding sequence. Plasmid pZ3 (described in detail above) was digested with Bam HI and Nco I to isolate the 1.25 kb ADH2-4^{c} promoter fragment. The 1.25 kb Bam HI-Nco I promoter fragment and the Nco I-Hind III fibronectin coding sequence fragment were ligated to Bam HI-Hind III linearized pUC19 to form plasmid pFN5.

Plasmid pFN5 was digested with Bam HI and Hind III to isolate the 2.1 kb promoter-fibronectin fragment. Plasmid pFN7 was digested with Hind III and Xba I to isolate the 2.8 kb fibronectin fragment that was modified to encode a stop codon following the Pro-Phe sequence. The TPI1 terminator sequence was obtained from pTT1 as a 0.7 kb Xba I-Sal I fragment. The 2.1 kb Bam HI-Hind III promoter-fibronectin fragment, the 2.8 kb Hind III-Xba I fibronectin fragment and the 0.7 kb TPI1 terminator fragment were joined in a four-part ligation with Bam HI-Xho I linearized pRPOT. A plasmid containing the fibronectin expression unit in the pRPOT vector was designated pR1.

The original clone pFH103 contained a frame-shift mutation in the EIIIB region of the fibronectin cDNA. The mutation was corrected by the replacement of the region with an analogous region from the plasmid pFHΔ3 (obtained from Jean Paul Thiery, Laboratoire de Physiopathologie du Developpement, CNRS URA 1337, Ecole Normale Superiure, 46 rue d'Ulm, 75230 Paris Cedex 05,

France). Plasmid pFHΔ3 was derived from pFH103 by excising the 3211 bp Xba I-Asp 718I fragment of fibronectin, blunting of the resultant adhesive ends and religating. Plasmid pFHΔ3 contains a DNA segment encoding the signal and propeptides, the first three and one half type I repeats, and the carboxy-terminal half of human fibronectin from the middle of the EIIIB segment.

Plasmid pR1 was digested with Bam HI and Kpn I to isolate the 2.2 kb promoter-fibronectin fragment. Plasmid pFHΔ3 was digested with Kpn I and Apa I to isolate the internal fibronectin fragment that corrects the frame-shift mutation present in the parent cDNA from pFH103. Plasmid pR1 was digested with Apa I and Bam HI to isolate the TPI1 terminator fragment. The 2.2 kb Bam HI-Kpn I promoter-fibronectin fragment, the 2.75 kb Kpn I-Apa I internal fibronectin fragment and the 0.69 kb Apa I-Bam HI TPI1 terminator fragment were joined in a four-part ligation with Bam HI-linearized pDPOT. The resulting construction was designated pD32.

A DNA segment encoding the ADH2-4^{c} promoter and initiation methionine from plasmid pD32 was subcloned into pIC19H (Marsh et al., Gene 32:481-486, 1984) as a 1.25 kb Bam HI-Nco I fragment. Plasmid pD32 was also digested with Nco I and Bgl II to isolate the 3 kb fibronectin cDNA fragment encoding amino acids 979-1972 of Sequence ID Number 2. The 1.25 kb Bam HI-Nco I fragment and the Nco I-Bgl II fragment were ligated with Bam HI-linearized pIC19H. A plasmid containing a Bam HI site proximal to the ADH2-4^{c} promoter was designated pFN14A.

B. - Construction of Plasmid pD38

An expression vector comprising a DNA segment encoding a fibronectin-fibrinogen hybrid protein operably linked to the ADH2-4^{c} promoter and the TPI1 terminator was constructed. To assemble the DNA sequence encoding the hybrid protein, a DNA segment encoding approximately the

carboxy-terminal 409 amino acids of the α chain of fibrinogen was first subcloned.

A fibrinogen α chain cDNA was obtained from Dominic W. Chung (Department of Biochemistry, University of Washington, Seattle, WA) in plasmid pHIα3 (Rixon et al., Biochemistry 22: 3250-3256, 1983). Sequence analysis of the cDNA insert in plasmid pHIα-3 revealed a deletion of codons 1348-1350 of the published sequence resulting in the deletion of Serine, amino acid 417.

The DNA segment encoding the carboxy-terminus of the fibrinogen α chain was subcloned into plasmid pUC19. Plasmid pHIα-3 was digested with Asp 718 and Ssp I to isolate the approximately 2 kb fragment encoding the carboxy-terminus of the fibrinogen α chain from amino acid 244 to amino acid 643 and some 3′ untranslated sequence of Sequence ID Number 4. Plasmid pTT1 was digested with Eco RV and Sal I to isolate the approximately 700 bp TPI1 terminator fragment. The 2 kb fibrinogen α chain sequence and the TPI1 terminator sequence were ligated with pUC19 that had been linearized with Asp 718 and Sal I. The ligation mixture was transformed into E. coli, and plasmid DNA was prepared and analyzed by restriction endonuclease and DNA sequence analysis. DNA sequence analysis of a candidate clone revealed that the Sal I site joining the TPI1 terminator sequence and the pUC19 polylinker site was not present. Plasmid DNA from the candidate clone was digested with Asp 718 and Bam HI to liberate the approximately 1.9 kb fibrinogen-TPI1 terminator fragment.

To join the fibronectin coding sequence with the fibrinogen α chain sequence, synthetic oligonucleotides were synthesized to provide, when annealed, a Sal I-Asp 718 adapter encoding an internal Afl II restriction site, and a sequence encoding amino acids 1886 through 1903 of fibronectin (Sequence ID Number 2), a glycine residue and amino acids 235 through 243 of the fibrinogen α chain (Sequence ID Number 4). Oligonucleotides ZC3521 and ZC3522 (Sequence ID Nos. 13 and 14) were annealed. The

1.9 kb Asp 718-Bam HI fibrinogen-TPI1 terminator fragment and the Sal I-Asp 718 ZC3521/ZC3522 adapter (Sequence ID Nos. 13 and 14) were ligated with pUC19 that had been linearized with Sal I and Bam HI. The resultant plasmid was designated pFG4.

The DNA segment encoding the fibronectin-fibrinogen α chain sequence in plasmid pFG4 was joined with the DNA segment encoding the amino-terminal fibronectin sequence (from amino acid 989 to amino acid 1885 of Sequence ID Number 2) in plasmid pFN14A to construct plasmid pD37. Plasmid pFN14A was digested with Bgl II and Afl II to isolate the approximately 3.9 kb ADH2-4^{C} promoter-fibronectin fragment. Plasmid pFG4 was digested with Afl II and Bam HI to isolate the approximately 2 kb fibronectin-fibrinogen-TPI1 terminator fragment. The 3.9 kb Bgl II-Afl II fragment and the 2 kb Afl II-Bam HI fragment were ligated with Bam HI-linearized pDPOT. A plasmid with the expression unit inserted with the direction of transcription in the same direction as the POT1 gene in the pDPOT vector was designated pD37.

To place the expression unit present in pD37 in the opposite orientation, such that the direction of transcription of the expression unit was in the opposite direction to that of the POT1 gene, plasmid pD37 was digested with Nco I and Xba I to isolate the approximately 4 kb fibronectin-fibrinogen α chain fragment. Plasmid pFN14A was digested with Bam HI and Nco I to isolate the approximately 1.3 kb ADH2-4^{C} promoter fragment. Plasmid pTT1 was digested with Bam HI and Xba I to isolate the approximately 700 bp TPI1 terminator fragment. The Bam HI-Nco I ADH2-4^{C} promoter fragment, the Nco I-Xba I fibronectin-fibrinogen α chain fragment and the Xba I-Bam HI TPI1 terminator fragment were ligated with Bam HI-linearized pDPOT that had been treated with calf alkaline phosphatase to prevent recircularization. A plasmid containing the expression unit in the opposite orientation relative to the POT1 gene was designated pD38. The

nucleotide sequence and deduced amino acid sequence of the DNA segment encoding the fibronectin-fibrinogen hybrid of plasmid pD38 is shown in Sequence ID Number 5. Plasmid pD38 was deposited on December 15, 1992 with the American Type Culture Collection (12301 Parklawn Drive, Rockville, MD) as an E. coli transformant.

Example 5 - Expression of a Fibronectin-Fibrinogen Hybrid Protein in Yeast

Plasmid pD38 was transformed into the Saccharomyces cerevisiae host strain ZM118 (MATa/MATα ura3/ura3 Δtpi1::URA3/Δtpi1::URA3 leu2-3,112/leu2-3,112 bar1/bar1 pep4::URA3/pep4::URA3 [cir^{o}]) using essentially the method described by Hinnen et al. (Proc. Natl. Acad. Sci. USA 75: 1929-1933, 1978). Transformants were selected for their ability to grow on medium containing glucose as the sole carbon source.

The ZM118[pD38] transformant was scaled up in a 60 liter fermenter to facilitate purification of the hybrid protein. A single ZM118[pD38] colony was selected from a YEPD + Ade + Leu plate (Table 1) and inoculated into -LeuTrpThrD medium (Table 1). The culture was incubated for approximately 52 hours after which the cells were harvested. The cells were washed in T.E. buffer (Sambrook et al., ibid.), resuspended in T.E. buffer + 30% glycerol, and aliquotted into 1 ml seed vials. The seed vials were stored at -80°C. One seed vial was used to inoculate 100 ml of YEPD + Ade + Leu (Table 1). The culture was grown for approximately 28 hours to a final A_{660} of 7.7. The 100 ml culture of ZM118[pD38] was inoculated into a 10 liter fermenter with a final volume of 6.0 liters of medium containing 10 g/L $(NH_4)_2SO_4$, 5 g/L KH_2PO_4, 5 g/L $MgSO_4 \cdot 7H_2O$, 1 g/L NaCl, 0.5 g/L $CaCl_2 \cdot 2H_2O$, 3.68 g/L A.A.I. (Table 1), 4.2 g/L citric acid, 60 g/L glucose, 10 ml/L Trace Metal Solution (Table 1), 0.4 ml/L PPG-2025 (Polypropylene glycol, MW 2025, Union Carbide Corp, Danbury, CT) that had been pH adjusted to pH 5.0

with NaOH. In addition to the inoculation culture, 30 ml of Vitamin solution was added (Table 1). The culture was grown for 23 hours at 30°C with the addition of 2 M NaOH to maintain pH of approximately 5.

Table 1
Media Recipes

-LeuThrTrp Amino Acid Mixture

4 g	adenine
3 g	L-arginine
5 g	L-aspartic acid
2 g	L-histidine free base
6 g	L-isoleucine
4 g	L-lysine-mono hydrochloride
2 g	L-methionine
6 g	L-phenylalanine
5 g	L-serine
5 g	L-tyrosine
4 g	uracil
6 g	L-valine

Mix all the ingredients and grind with a mortar and pestle until the mixture is finely ground.

-LeuTrpThrD

20 g	glucose
6.7 g	Yeast Nitrogen Base without amino acids (DIFCO Laboratories, Detroit, MI)
0.6 g	-LeuThrTrp Amino Acid Mixture
18 g	Agar

Mix all the ingredients in distilled water. Add distilled water to a final volume of 1 liter. Autoclave 15 minutes. Pour plates and allow to solidify.

Table 1 continued

YEPD + Ade + Leu Plates

20 g	glucose
20 g	Bacto Peptone (DIFCO Laboratories)
10 g	Bacto Yeast Extract (DIFCO Laboratories)
18 g	agar
4 ml	1% adenine
8 ml	1% L-leucine

Mix all ingredients in distilled water, and bring to a final volume of 1 liter. Autoclave 25 minutes and pour plates.

YEPD + Ade + Leu Medium

20 g	glucose
20 g	Bacto Peptone (DIFCO Laboratories)
10 g	Bacto Yeast Extract (DIFCO Laboratories)
4 ml	1% adenine
8 ml	1% L-leucine

Mix all ingredients in distilled water, and bring to a final volume of 1 liter. Autoclave 25 minutes.

Table 1 continued

	A.A.I.
4.0 g	adenine
5.0 g	L-alanine
2.0 g	L-arginine
5.0 g	L-asparagine
5.0 g	L-aspartic acid
5.0 g	L-cysteine
5.0 g	L-glutamine
5.0 g	L-glutamic acid
5.0 g	L-glycine
8.0 g	L-histidine
5.0 g	L-isoleucine
3.0 g	L-lysine-mono hydrochloride
2.0 g	L-methionine
5.0 g	L-phenylalanine
5.0 g	L-proline
5.0 g	L-serine
5.0 g	L-threonine
2.0 g	L-tryptophan
3.0 g	L-tyrosine
3.0 g	uracil
5.0 g	L-valine

Mix all the ingredients and grind with a mortar and pestle until the mixture is finely ground. Store at room temperature.

Table 1 continued

Trace Metal Solution

0.68 g	$ZnCl_2$
5.4 g	$FeCl_3 \cdot 6H_2O$
1.91 g	$MnCl_2 \cdot 4H_2O$
0.22 g	$CuSO_4 \cdot 5H_2O$
0.258 g	$CoCl_2$
0.062 g	H_3BO_3
0.002 g	$(NH_4)_6Mo_2O_2$
0.002 g	KI
10.0 ml	37% HCl

Dissolve solids in water and bring to a final volume of 1 liter.

Vitamin Solution

25 mg	d-biotin
400 mg	thiamine
400 mg	pyridoxine
7.5 g	meso-inositol
7.5 g	Ca pantothenate
300 mg	niacinamide
50 mg	folic acid
100 mg	riboflavin
500 mg	choline

Dissolve solids in water and bring to a final volume of 1 liter.

A 60 liter fermenter with a final volume of 50 liters of medium containing 60 g/L yeast extract (Universal Foods, Milwaukee, WI), 2.5 g/L $MgSO_4 \cdot 7H_2O$ (Mallinkrodt Inc., St. Louis, MO), 1 g/L $CaCl_2 \cdot 2H_2O$ (Mallinkrodt, Inc.), 1 g/L KCl (Mallinkrodt, Inc.), 10 ml/L of Trace Metal Solution (Table 1), 0.5 ml/L PPG-2025 (Union Carbide) that had been adjusted to a pH of 5.0 with

H_3PO_4 was prepared, and the medium was sterilized. After sterilization, 5.0 liters of the 23 hour fermentation culture and 500 ml of Vitamin Solution (Table 1) were inoculated into the medium. During the fermentation, a solution of 50% glucose, 5% $(NH_4)_2SO_4$, 0.05% citric acid was fed into the fermenter at a rate of 150 ml/hour, and the pH was maintained at approximately pH 5 by the addition of 2 M NH_4OH. PPG-2025 was added as needed to control foaming. At approximately 49 hours post inoculation, an ethanol feed was begun by the addition of ethanol to the fermenter at a rate of 150 ml/min. The culture was grown for a total of 67.25 hours at 30°C.

At the end of the fermentation, 50 liters of the culture was diluted to 100 liters with water. The cells were removed from the spent medium by centrifuging 50 liters at a time through a Westfalia CSA 19 centrifuge (Westfalia, Oelde, Germany) at a flow rate of 4 liters/min. The cells were rinsed with water. From the centrifugation, approximately 20 liters of cell slurry containing approximately 35% cells was obtained. Salts were added to the slurry to achieve a final concentration of the following salts: 50 mM NaCl, 10 mM Na_2HPO_4, 5 mM EDTA. The cell slurry was passed through a Dynomill bead mill using 0.5 mm lead-free glass beads (Willy A Bachofen AG MashinenFabrik, Basle, Switzerland) at a rate of 4 liters per minute. The Dynomill was rinsed with Lysis buffer (50 mM NaCl, 10 mM Na_2HPO_4, 5 mM EDTA, pH 7.2) to a final volume of 80 liters. The final slurry had a pH of 6.8, a temperature of approximately 10°C and a conductivity of 5 ms/cm.

The cell slurry was subjected to centrifugation as described above, and the cell pellet was rinsed with lysis buffer. After centrifugation approximately 20 liters of cell slurry was obtained. The cell slurry was extracted by first adjusting the concentration of the cell debris to approximately 40-50% with lysis buffer. Solid urea, NaCl and EDTA were added to the cell slurry to

achieve a final concentration of approximately 8 M urea, 0.3 M NaCl and 10 mM EDTA. The approximate salt concentrations were obtained by the addition of 450 g/L of urea, 18 g/L of NaCl and 4.2 g/L of EDTA. The cell slurry was adjusted to pH 7.8 with 0.5 M NaOH. The solids were dissolved into the slurry and the pellets were extracted for a total of 50 minutes. Following extraction, the mixture was diluted 1 to 4 with water, adjusted to a conductivity of 12.5 ms/cm with NaCl and adjusted to a pH of 9.5 with 0.5 M NaOH.

The extracted slurry was centrifuged as described above with the lysis buffer rinse. The pH of the supernatant was adjusted to pH 9.5 with 0.5 M NaOH. The supernatant was analyzed by SDS polyacrylamide gel electrophoresis (SDS-PAGE) using the PHAST System Separation and Control Unit (Pharmacia LKB Biotechnology Inc., Piscataway, NJ), and the protein was visualized using Coomassie Blue staining. A 2 liter Q-sepharose column (Pharmacia) was equilibrated at 5 liters/hour with successive washes of the following solutions: 8 liters of 3 M urea, 1 M NaCl, 50 mM glycine, pH 11.5; 5 liters of 0.5 M NaOH; 1.5 liters of water; 5 liters of 0.1 M HCl; and 6.0 liters of Wash buffer (50 mM glycine, 90 mM NaCl, pH 9.5 with a conductivity of 12.5 ms/cm). The supernatant (110 liters) was then applied to the column at 5 liters per hour.

The column ran dry after loading the supernatant. The gel was resuspended in Wash buffer and repacked. The repacked column was washed with 4 liters of 50 mM glycine, 90 mM NaCl, 5 mM EDTA, pH 10.0. The material was eluted with elution buffer (50 mM glycine, 5 mM EDTA (pH 9.9) with a final concentration of NaCl giving a conductivity of 30.2 cm/ms (approximately 270 mM NaCl)) at 100 ml per minute. The approximately 600 ml fractions were collected after the conductivity of the eluant reached the conductivity of the elution buffer. Fractions

were analyzed by SDS-PAGE analysis as described above and fractions 1 through 10 were pooled.

The pooled fractions were then applied to a 2 liter phenyl Sepharose column (Pharmacia) that had been equilibrated by successive washes at 5 liters per hour with the following solutions: 3 liters of 0.5 M NaOH; 3 liters of water; 3 liters of 2 M urea, 50 mM glycine, pH 10.5; 1.5 liters of water; 3 liters of 0.1 M HCl; and 3 liters of Equilibration buffer (50 mM glycine, 2.5 M NaCl, 2 mM EDTA (pH 10.0) with a conductivity of 180 ms/cm). The pooled peak fractions, which had been adjusted to a conductivity of 180 ms/cm with NaCl and a pH of 10.0 with 0.5 M NaOH, were loaded onto the phenyl sepharose column. Following the loading of the peak fractions, the column was washed with Equilibration buffer. The column was eluted with 6 liters of 50 mM glycine, 2 mM EDTA (pH 10.25) with a NaCl concentration giving the solution a conductivity of 96 ms/cm. The conductivity of the eluant was measured throughout the elution. The conductivity of the eluant upon starting the elution was 180 ms/cm. In the third fraction, the conductivity of the eluant dropped to 96 ms/cm. At this point, the elution buffer was changed to a buffer having the conductivity of 42 ms/cm. The eluant was collected through fraction number 8.

Example 6 - Cross-Linking Assay Using the Hybrid Fibrinogen-Fibronectin Protein

The ability of the purified fibrinogen-fibronectin hybrid protein to form transglutminase-catalyzed interchain cross links was assessed. The transglutaminase activity was provided by the addition of recombinant factor XIII and thrombin or by the addition of recombinant factor XIIIa.

A. Preparation of Factor XIII

Recombinant factor XIII was prepared essentially as described in co-pending U.S. Patent Application No. 07/927,196, which is incorporated by reference herein in its entirety. Briefly, factor XIII was isolated from a strain of the yeast Saccharomyces cerevisiae that had been transformed with an expression vector capable of directing the expression of factor XIII. The factor XIII-producing cells were harvested and lysed, and a cleared lysate was prepared. The lysate was fractionated by anion exchange chromatography at neutral to slightly alkaline pH using a column of derivatized agarose, such as DEAE FAST-FLOW SEPHAROSE (Pharmacia LKB Biotechnology, Piscataway, NJ) or the like. Factor XIII was then precipitated from the column eluate by concentrating the eluate and adjusting the pH to between 5.2 and 5.5, such as by diafiltration against ammonium succinate buffer. The precipitate was then dissolved and further purified using conventional chromatographic techniques, such as gel filtration and hydrophobic interaction chromatography. The purified factor XIII was dialyzed, filtered, aliquotted and lyophilized. The factor XIIIa content was determined (Bishop et al., Biochemistry 29: 1861-1869, 1990, which is incorporated by reference herein in its entirety) by fluorometric assay of the dissolved, thrombin-activated material.

Factor XIII was activated to factor XIIIa by adding 2 U of thrombin per 100 mg of factor XIII. The

factor XIII was dissolved in buffer (20 mM sodium borate (pH 8.3), 1 mM $CaCl_2$). The thrombin was added, and the reaction was incubated at room temperature for twenty minutes.

B. Cross-Linking Assays

The level of cross-linking between the hybrid proteins was measured as a rise in the absorbance at 350 nm over time in reaction mixtures containing the hybrid protein, factor XIII and thrombin or the hybrid protein and factor XIIIa. Control reactions were prepared containing factor XIII and thrombin or factor XIIIa alone. Cross-linking reactions were carried out in 1 ml cuvettes. For cross-linking reactions containing factor XIII and thrombin, each reaction mixture was set up by placing 110 µl containing 40 Units of factor XIII, 36.7 µl containing 13 Units of factor XIII or 12.2 µl containing 4 Units of factor XIII (described above) in one corner of the cuvette and 20 µl containing 4 Units of thrombin (Sigma) in the opposite corner such that the solutions were not mixed. The reaction was initiated by the addition of 1 ml of 2 mg/ml hybrid protein in buffer (10 mM Tris (pH 7.6), 20 mM sodium borate, 140 mM NaCl, 10 mM $CaCl_2$). The absorbance of each reaction was read at 350 nm with the addition of protein being the first absorbance point. For cross-linking reactions containing factor XIIIa, each reaction was set up by placing 110 µl containing 40 Units of factor XIIIa, 36.7 µl containing 13 Units of factor XIIIa or 12.2 µl containing 4 Units of factor XIIIa in the cuvette and adding 1 ml of 2 mg/ml hybrid in buffer (10 mM Tris (pH 7.6), 140 mM NaCl, 10 mM $CaCl_2$). The absorbance of the solution was read at 350 nm as described above. Analysis of the data generated from the absorbance time courses showed a sharp increase in absorbance in the presence of hybrid protein and the active transglutaminase relative to the rise in absorbance in the absence of hybrid protein

(Figures 2-5). The results indicated that the hybrid protein is capable of transglutaminase-induced cross-linking.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviation from the spirit and scope of the invention. Accordingly, the invention is not to be limited except as by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Irani, Meher H.

(ii) TITLE OF INVENTION: HYBRID CROSS-LINKING PROTEINS

(iii) NUMBER OF SEQUENCES: 14

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: ZymoGenetics, Inc.
(B) STREET: 4225 Roosevelt Way, N.E.
(C) CITY: Seattle
(D) STATE: WA
(E) COUNTRY: USA
(F) ZIP: 98105

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: WO
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/998,271
(B) FILING DATE: 31-DEC-1992

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Parker, Gary E
(B) REGISTRATION NUMBER: 31-648
(C) REFERENCE/DOCKET NUMBER: 92-26PC

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 206-547-8080 ext 322
(B) TELEFAX: 206-548-2329

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7803 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS

(B) LOCATION: 6..7346

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
TCAAC ATG CTT AGG GGT CCG GGG CCC GGG CTG CTG CTG CTG GCC GTC          47
      Met Leu Arg Gly Pro Gly Pro Gly Leu Leu Leu Leu Ala Val
        1               5                  10

CTG TGC CTG GGG ACA GCG GTG CCC TCC ACG GGA GCC TCG AAG AGC AAG        95
Leu Cys Leu Gly Thr Ala Val Pro Ser Thr Gly Ala Ser Lys Ser Lys
 15                  20                  25                  30

AGG CAG GCT CAG CAA ATG GTT CAG CCC CAG TCC CCG GTG GCT GTC AGT       143
Arg Gln Ala Gln Gln Met Val Gln Pro Gln Ser Pro Val Ala Val Ser
                35                  40                  45

CAA AGC AAG CCC GGT TGT TAT GAC AAT GGA AAA CAC TAT CAG ATA AAT       191
Gln Ser Lys Pro Gly Cys Tyr Asp Asn Gly Lys His Tyr Gln Ile Asn
            50                  55                  60

CAA CAG TGG GAG CGG ACC TAC CTA GGT AAT GTG TTG GTT TGT ACT TGT       239
Gln Gln Trp Glu Arg Thr Tyr Leu Gly Asn Val Leu Val Cys Thr Cys
        65                  70                  75

TAT GGA GGA AGC CGA GGT TTT AAC TGC GAA AGT AAA CCT GAA GCT GAA       287
Tyr Gly Gly Ser Arg Gly Phe Asn Cys Glu Ser Lys Pro Glu Ala Glu
    80                  85                  90

GAG ACT TGC TTT GAC AAG TAC ACT GGG AAC ACT TAC CGA GTG GGT GAC       335
Glu Thr Cys Phe Asp Lys Tyr Thr Gly Asn Thr Tyr Arg Val Gly Asp
 95                 100                 105                 110

ACT TAT GAG CGT CCT AAA GAC TCC ATG ATC TGG GAC TGT ACC TGC ATC       383
Thr Tyr Glu Arg Pro Lys Asp Ser Met Ile Trp Asp Cys Thr Cys Ile
                115                 120                 125

GGG GCT GGG CGA GGG AGA ATA AGC TGT ACC ATC GCA AAC CGC TGC CAT       431
Gly Ala Gly Arg Gly Arg Ile Ser Cys Thr Ile Ala Asn Arg Cys His
            130                 135                 140

GAA GGG GGT CAG TCC TAC AAG ATT GGT GAC ACC TGG AGG AGA CCA CAT       479
Glu Gly Gly Gln Ser Tyr Lys Ile Gly Asp Thr Trp Arg Arg Pro His
        145                 150                 155

GAG ACT GGT GGT TAC ATG TTA GAG TGT GTG TGT CTT GGT AAT GGA AAA       527
Glu Thr Gly Gly Tyr Met Leu Glu Cys Val Cys Leu Gly Asn Gly Lys
    160                 165                 170

GGA GAA TGG ACC TGC AAG CCC ATA GCT GAG AAG TGT TTT GAT CAT GCT       575
Gly Glu Trp Thr Cys Lys Pro Ile Ala Glu Lys Cys Phe Asp His Ala
175                 180                 185                 190

GCT GGG ACT TCC TAT GTG GTC GGA GAA ACG TGG GAG AAG CCC TAC CAA       623
Ala Gly Thr Ser Tyr Val Val Gly Glu Thr Trp Glu Lys Pro Tyr Gln
                195                 200                 205
```

```
GGC TGG ATG ATG GTA GAT TGT ACT TGC CTG GGA GAA GGC AGC GGA CGC        671
Gly Trp Met Met Val Asp Cys Thr Cys Leu Gly Glu Gly Ser Gly Arg
            210                 215                 220

ATC ACT TGC ACT TCT AGA AAT AGA TGC AAC GAT CAG GAC ACA AGG ACA        719
Ile Thr Cys Thr Ser Arg Asn Arg Cys Asn Asp Gln Asp Thr Arg Thr
        225                 230                 235

TCC TAT AGA ATT GGA GAC ACC TGG AGC AAG AAG GAT AAT CGA GGA AAC        767
Ser Tyr Arg Ile Gly Asp Thr Trp Ser Lys Lys Asp Asn Arg Gly Asn
    240                 245                 250

CTG CTC CAG TGC ATC TGC ACA GGC AAC GGC CGA GGA GAG TGG AAG TGT        815
Leu Leu Gln Cys Ile Cys Thr Gly Asn Gly Arg Gly Glu Trp Lys Cys
255                 260                 265                 270

GAG AGG CAC ACC TCT GTG CAG ACC ACA TCG AGC GGA TCT GGC CCC TTC        863
Glu Arg His Thr Ser Val Gln Thr Thr Ser Ser Gly Ser Gly Pro Phe
                275                 280                 285

ACC GAT GTT CGT GCA GCT GTT TAC CAA CCG CAG CCT CAC CCC CAG CCT        911
Thr Asp Val Arg Ala Ala Val Tyr Gln Pro Gln Pro His Pro Gln Pro
            290                 295                 300

CCT CCC TAT GGC CAC TGT GTC ACA GAC AGT GGT GTG GTC TAC TCT GTG        959
Pro Pro Tyr Gly His Cys Val Thr Asp Ser Gly Val Val Tyr Ser Val
        305                 310                 315

GGG ATG CAG TGG TTG AAG ACA CAA GGA AAT AAG CAA ATG CTT TGC ACG       1007
Gly Met Gln Trp Leu Lys Thr Gln Gly Asn Lys Gln Met Leu Cys Thr
    320                 325                 330

TGC CTG GGC AAC GGA GTC AGC TGC CAA GAG ACA GCT GTA ACC CAG ACT       1055
Cys Leu Gly Asn Gly Val Ser Cys Gln Glu Thr Ala Val Thr Gln Thr
335                 340                 345                 350

TAC GGT GGC AAC TTA AAT GGA GAG CCA TGT GTC TTA CCA TTC ACC TAC       1103
Tyr Gly Gly Asn Leu Asn Gly Glu Pro Cys Val Leu Pro Phe Thr Tyr
                355                 360                 365

AAT GGC AGG ACG TTC TAC TCC TGC ACC ACG GAA GGG CGA CAG GAC GGA       1151
Asn Gly Arg Thr Phe Tyr Ser Cys Thr Thr Glu Gly Arg Gln Asp Gly
            370                 375                 380

CAT CTT TGG TGC AGC ACA ACT TCG AAT TAT GAG CAG GAC CAG AAA TAC       1199
His Leu Trp Cys Ser Thr Thr Ser Asn Tyr Glu Gln Asp Gln Lys Tyr
        385                 390                 395

TCT TTC TGC ACA GAC CAC ACT GTT TTG GTT CAG ACT CAA GGA GGA AAT       1247
Ser Phe Cys Thr Asp His Thr Val Leu Val Gln Thr Gln Gly Gly Asn
    400                 405                 410

TCC AAT GGT GCC TTG TGC CAC TTC CCC TTC CTA TAC AAC AAC CAC AAT       1295
Ser Asn Gly Ala Leu Cys His Phe Pro Phe Leu Tyr Asn Asn His Asn
415                 420                 425                 430
```

```
TAC ACT GAT TGC ACT TCT GAG GGC AGA AGA GAC AAC ATG AAG TGG TGT      1343
Tyr Thr Asp Cys Thr Ser Glu Gly Arg Arg Asp Asn Met Lys Trp Cys
                435                 440                 445

GGG ACC ACA CAG AAC TAT GAT GCC GAC CAG AAG TTT GGG TTC TGC CCC      1391
Gly Thr Thr Gln Asn Tyr Asp Ala Asp Gln Lys Phe Gly Phe Cys Pro
            450                 455                 460

ATG GCT GCC CAC GAG GAA ATC TGC ACA ACC AAT GAA GGG GTC ATG TAC      1439
Met Ala Ala His Glu Glu Ile Cys Thr Thr Asn Glu Gly Val Met Tyr
        465                 470                 475

CGC ATT GGA GAT CAG TGG GAT AAG CAG CAT GAC ATG GGT CAC ATG ATG      1487
Arg Ile Gly Asp Gln Trp Asp Lys Gln His Asp Met Gly His Met Met
    480                 485                 490

AGG TGC ACG TGT GTT GGG AAT GGT CGT GGG GAA TGG ACA TGC ATT GCC      1535
Arg Cys Thr Cys Val Gly Asn Gly Arg Gly Glu Trp Thr Cys Ile Ala
495                 500                 505                 510

TAC TCG CAA CTT CGA GAT CAG TGC ATT GTT GAT GAC ATC ACT TAC AAT      1583
Tyr Ser Gln Leu Arg Asp Gln Cys Ile Val Asp Asp Ile Thr Tyr Asn
                515                 520                 525

GTG AAC GAC ACA TTC CAC AAG CGT CAT GAA GAG GGG CAC ATG CTG AAC      1631
Val Asn Asp Thr Phe His Lys Arg His Glu Glu Gly His Met Leu Asn
            530                 535                 540

TGT ACA TGC TTC GGT CAG GGT CGG GGC AGG TGG AAG TGT GAT CCC GTC      1679
Cys Thr Cys Phe Gly Gln Gly Arg Gly Arg Trp Lys Cys Asp Pro Val
        545                 550                 555

GAC CAA TGC CAG GAT TCA GAG ACT GGG ACG TTT TAT CAA ATT GGA GAT      1727
Asp Gln Cys Gln Asp Ser Glu Thr Gly Thr Phe Tyr Gln Ile Gly Asp
    560                 565                 570

TCA TGG GAG AAG TAT GTG CAT GGT GTC AGA TAC CAG TGC TAC TGC TAT      1775
Ser Trp Glu Lys Tyr Val His Gly Val Arg Tyr Gln Cys Tyr Cys Tyr
575                 580                 585                 590

GGC CGT GGC ATT GGG GAG TGG CAT TGC CAA CCT TTA CAG ACC TAT CCA      1823
Gly Arg Gly Ile Gly Glu Trp His Cys Gln Pro Leu Gln Thr Tyr Pro
                595                 600                 605

AGC TCA AGT GGT CCT GTC GAA GTA TTT ATC ACT GAG ACT CCG AGT CAG      1871
Ser Ser Ser Gly Pro Val Glu Val Phe Ile Thr Glu Thr Pro Ser Gln
            610                 615                 620

CCC AAC TCC CAC CCC ATC CAG TGG AAT GCA CCA CAG CCA TCT CAC ATT      1919
Pro Asn Ser His Pro Ile Gln Trp Asn Ala Pro Gln Pro Ser His Ile
        625                 630                 635
```

```
TCC AAG TAC ATT CTC AGG TGG AGA CCT AAA AAT TCT GTA GGC CGT TGG      1967
Ser Lys Tyr Ile Leu Arg Trp Arg Pro Lys Asn Ser Val Gly Arg Trp
    640                 645                 650

AAG GAA GCT ACC ATA CCA GGC CAC TTA AAC TCC TAC ACC ATC AAA GGC      2015
Lys Glu Ala Thr Ile Pro Gly His Leu Asn Ser Tyr Thr Ile Lys Gly
655                 660                 665                 670

CTG AAG CCT GGT GTG GTA TAC GAG GGC CAG CTC ATC AGC ATC CAG CAG      2063
Leu Lys Pro Gly Val Val Tyr Glu Gly Gln Leu Ile Ser Ile Gln Gln
                675                 680                 685

TAC GGC CAC CAA GAA GTG ACT CGC TTT GAC TTC ACC ACC ACC AGC ACC      2111
Tyr Gly His Gln Glu Val Thr Arg Phe Asp Phe Thr Thr Thr Ser Thr
            690                 695                 700

AGC ACA CCT GTG ACC AGC AAC ACC GTG ACA GGA GAG ACG ACT CCC TTT      2159
Ser Thr Pro Val Thr Ser Asn Thr Val Thr Gly Glu Thr Thr Pro Phe
        705                 710                 715

TCT CCT CTT GTG GCC ACT TCT GAA TCT GTG ACC GAA ATC ACA GCC AGT      2207
Ser Pro Leu Val Ala Thr Ser Glu Ser Val Thr Glu Ile Thr Ala Ser
    720                 725                 730

AGC TTT GTG GTC TCC TGG GTC TCA GCT TCC GAC ACC GTG TCG GGA TTC      2255
Ser Phe Val Val Ser Trp Val Ser Ala Ser Asp Thr Val Ser Gly Phe
735                 740                 745                 750

CGG GTG GAA TAT GAG CTG AGT GAG GAG GGA GAT GAG CCA CAG TAC CTG      2303
Arg Val Glu Tyr Glu Leu Ser Glu Glu Gly Asp Glu Pro Gln Tyr Leu
                755                 760                 765

GAT CTT CCA AGC ACA GCC ACT TCT GTG AAC ATC CCT GAC CTG CTT CCT      2351
Asp Leu Pro Ser Thr Ala Thr Ser Val Asn Ile Pro Asp Leu Leu Pro
            770                 775                 780

GGC CGA AAA TAC ATT GTA AAT GTC TAT CAG ATA TCT GAG GAT GGG GAG      2399
Gly Arg Lys Tyr Ile Val Asn Val Tyr Gln Ile Ser Glu Asp Gly Glu
        785                 790                 795

CAG AGT TTG ATC CTG TCT ACT TCA CAA ACA ACA GCG CCT GAT GCC CCT      2447
Gln Ser Leu Ile Leu Ser Thr Ser Gln Thr Thr Ala Pro Asp Ala Pro
    800                 805                 810

CCT GAC CCG ACT GTG GAC CAA GTT GAT GAC ACC TCA ATT GTT GTT CGC      2495
Pro Asp Pro Thr Val Asp Gln Val Asp Asp Thr Ser Ile Val Val Arg
815                 820                 825                 830

TGG AGC AGA CCC CAG GCT CCC ATC ACA GGG TAC AGA ATA GTC TAT TCG      2543
Trp Ser Arg Pro Gln Ala Pro Ile Thr Gly Tyr Arg Ile Val Tyr Ser
                835                 840                 845

CCA TCA GTA GAA GGT AGC AGC ACA GAA CTC AAC CTT CCT GAA ACT GCA      2591
Pro Ser Val Glu Gly Ser Ser Thr Glu Leu Asn Leu Pro Glu Thr Ala
            850                 855                 860
```

```
AAC TCC GTC ACC CTC AGT GAC TTG CAA CCT GGT GTT CAG TAT AAC ATC      2639
Asn Ser Val Thr Leu Ser Asp Leu Gln Pro Gly Val Gln Tyr Asn Ile
        865                 870                 875

ACT ATC TAT GCT GTG GAA GAA AAT CAA GAA AGT ACA CCT GTT GTC ATT      2687
Thr Ile Tyr Ala Val Glu Glu Asn Gln Glu Ser Thr Pro Val Val Ile
    880                 885                 890

CAA CAA GAA ACC ACT GGC ACC CCA CGC TCA GAT ACA GTG CCC TCT CCC      2735
Gln Gln Glu Thr Thr Gly Thr Pro Arg Ser Asp Thr Val Pro Ser Pro
895                 900                 905                 910

AGG GAC CTG CAG TTT GTG GAA GTG ACA GAC GTG AAG GTC ACC ATC ATG      2783
Arg Asp Leu Gln Phe Val Glu Val Thr Asp Val Lys Val Thr Ile Met
                915                 920                 925

TGG ACA CCG CCT GAG AGT GCA GTG ACC GGC TAC CGT GTG GAT GTG ATC      2831
Trp Thr Pro Pro Glu Ser Ala Val Thr Gly Tyr Arg Val Asp Val Ile
            930                 935                 940

CCC GTC AAC CTG CCT GGC GAG CAC GGG CAG AGG CTG CCC ATC AGC AGG      2879
Pro Val Asn Leu Pro Gly Glu His Gly Gln Arg Leu Pro Ile Ser Arg
        945                 950                 955

AAC ACC TTT GCA GAA GTC ACC GGG CTG TCC CCT GGG GTC ACC TAT TAC      2927
Asn Thr Phe Ala Glu Val Thr Gly Leu Ser Pro Gly Val Thr Tyr Tyr
    960                 965                 970

TTC AAA GTC TTT GCA GTG AGC CAT GGG AGG GAG AGC AAG CCT CTG ACT      2975
Phe Lys Val Phe Ala Val Ser His Gly Arg Glu Ser Lys Pro Leu Thr
975                 980                 985                 990

GCT CAA CAG ACA ACC AAA CTG GAT GCT CCC ACT AAC CTC CAG TTT GTC      3023
Ala Gln Gln Thr Thr Lys Leu Asp Ala Pro Thr Asn Leu Gln Phe Val
                995                 1000                1005

AAT GAA ACT GAT TCT ACT GTC CTG GTG AGA TGG ACT CCA CCT CGG GCC      3071
Asn Glu Thr Asp Ser Thr Val Leu Val Arg Trp Thr Pro Pro Arg Ala
            1010                1015                1020

CAG ATA ACA GGA TAC CGA CTG ACC GTG GGC CTT ACC CGA AGA GGC CAG      3119
Gln Ile Thr Gly Tyr Arg Leu Thr Val Gly Leu Thr Arg Arg Gly Gln
        1025                1030                1035

CCC AGG CAG TAC AAT GTG GGT CCC TCT GTC TCC AAG TAC CCC CTG AGG      3167
Pro Arg Gln Tyr Asn Val Gly Pro Ser Val Ser Lys Tyr Pro Leu Arg
    1040                1045                1050

AAT CTG CAG CCT GCA TCT GAG TAC ACC GTA TCC CTC GTG GCC ATA AAG      3215
Asn Leu Gln Pro Ala Ser Glu Tyr Thr Val Ser Leu Val Ala Ile Lys
1055                1060                1065                1070
```

```
GGC AAC CAA GAG AGC CCC AAA GCC ACT GGA GTC TTT ACC ACA CTG CAG      3263
Gly Asn Gln Glu Ser Pro Lys Ala Thr Gly Val Phe Thr Thr Leu Gln
                1075                1080                1085

CCT GGG AGC TCT ATT CCA CCT TAC AAC ACC GAG GTG ACT GAG ACC ACC      3311
Pro Gly Ser Ser Ile Pro Pro Tyr Asn Thr Glu Val Thr Glu Thr Thr
            1090                1095                1100

ATC GTG ATC ACA TGG ACG CCT GCT CCA AGA ATT GGT TTT AAG CTG GGT      3359
Ile Val Ile Thr Trp Thr Pro Ala Pro Arg Ile Gly Phe Lys Leu Gly
        1105                1110                1115

GTA CGA CCA AGC CAG GGA GGA GAG GCA CCA CGA GAA GTG ACT TCA GAC      3407
Val Arg Pro Ser Gln Gly Gly Glu Ala Pro Arg Glu Val Thr Ser Asp
    1120                1125                1130

TCA GGA AGC ATC GTT GTG TCC GGC TTG ACT CCA GGA GTA GAA TAC GTC      3455
Ser Gly Ser Ile Val Val Ser Gly Leu Thr Pro Gly Val Glu Tyr Val
1135                1140                1145                1150

TAC ACC ATC CAA GTC CTG AGA GAT GGA CAG GAA AGA GAT GCG CCA ATT      3503
Tyr Thr Ile Gln Val Leu Arg Asp Gly Gln Glu Arg Asp Ala Pro Ile
                1155                1160                1165

GTA AAC AAA GTG GTG ACA CCA TTG TCT CCA CCA ACA AAC TTG CAT CTG      3551
Val Asn Lys Val Val Thr Pro Leu Ser Pro Pro Thr Asn Leu His Leu
            1170                1175                1180

GAG GCA AAC CCT GAC ACT GGA GTG CTC ACA GTC TCC TGG GAG AGG AGC      3599
Glu Ala Asn Pro Asp Thr Gly Val Leu Thr Val Ser Trp Glu Arg Ser
        1185                1190                1195

ACC ACC CCA GAC ATT ACT GGT TAT AGA ATT ACC ACA ACC CCT ACA AAC      3647
Thr Thr Pro Asp Ile Thr Gly Tyr Arg Ile Thr Thr Thr Pro Thr Asn
    1200                1205                1210

GGC CAG CAG GGA AAT TCT TTG GAA GAA GTG GTC CAT GCT GAT CAG AGC      3695
Gly Gln Gln Gly Asn Ser Leu Glu Glu Val Val His Ala Asp Gln Ser
1215                1220                1225                1230

TCC TGC ACT TTT GAT AAC CTG AGT CCC GGC CTG GAG TAC AAT GTC AGT      3743
Ser Cys Thr Phe Asp Asn Leu Ser Pro Gly Leu Glu Tyr Asn Val Ser
                1235                1240                1245

GTT TAC ACT GTC AAG GAT GAC AAG GAA AGT GTC CCT ATC TCT GAT ACC      3791
Val Tyr Thr Val Lys Asp Asp Lys Glu Ser Val Pro Ile Ser Asp Thr
            1250                1255                1260

ATC ATC CCA GAG GTG CCC CAA CTC ACT GAC CTA AGC TTT GTT GAT ATA      3839
Ile Ile Pro Glu Val Pro Gln Leu Thr Asp Leu Ser Phe Val Asp Ile
        1265                1270                1275

ACC GAT TCA AGC ATC GGC CTG AGG TGG ACC CCG CTA AAC TCT TCC ACC      3887
Thr Asp Ser Ser Ile Gly Leu Arg Trp Thr Pro Leu Asn Ser Ser Thr
    1280                1285                1290
```

```
ATT ATT GGG TAC CGC ATC ACA GTA GTT GCG GCA GGA GAA GGT ATC CCT      3935
Ile Ile Gly Tyr Arg Ile Thr Val Val Ala Ala Gly Glu Gly Ile Pro
1295                1300                1305                1310

ATT TTT GAA GAT TTT GTG TAC TCC TCA GTA GGA TAC TAC ACA GTC ACA      3983
Ile Phe Glu Asp Phe Val Tyr Ser Ser Val Gly Tyr Tyr Thr Val Thr
                1315                1320                1325

GGG CTG GAG CCG GGC ATT GAC TAT GAT ATC AGC GTT ATC ACT CTC ATT      4031
Gly Leu Glu Pro Gly Ile Asp Tyr Asp Ile Ser Val Ile Thr Leu Ile
            1330                1335                1340

AAT GGC GGC GAG AGT GCC CCT ACT ACA CTG ACA CAA CAA ACG GCT GTT      4079
Asn Gly Gly Glu Ser Ala Pro Thr Thr Leu Thr Gln Gln Thr Ala Val
        1345                1350                1355

CCT CCT CCC ACT GAC CTG CGA TTC ACC AAC ATT GGT CCA GAC ACC ATG      4127
Pro Pro Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met
    1360                1365                1370

CGT GTC ACC TGG GCT CCA CCC CCA TCC ATT GAT TTA ACC AAC TTC CTG      4175
Arg Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
1375                1380                1385                1390

GTG CGT TAC TCA CCT GTG AAA AAT GAG GAA GAT GTT GCA GAG TTG TCA      4223
Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu Ser
                1395                1400                1405

ATT TCT CCT TCA GAC AAT GCA GTG GTC TTA ACA AAT CTC CTG CCT GGT      4271
Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu Pro Gly
            1410                1415                1420

ACA GAA TAT GTA GTG AGT GTC TCC AGT GTC TAC GAA CAA CAT GAG AGC      4319
Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln His Glu Ser
        1425                1430                1435

ACA CCT CTT AGA GGA AGA CAG AAA ACA GGT CTT GAT TCC CCA ACT GGC      4367
Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp Ser Pro Thr Gly
    1440                1445                1450

ATT GAC TTT TCT GAT ATT ACT GCC AAC TCT TTT ACT GTG CAC TGG ATT      4415
Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe Thr Val His Trp Ile
1455                1460                1465                1470

GCT CCT CGA GCC ACC ATC ACT GGC TAC AGG ATC CGC CAT CAT CCC GAG      4463
Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg Ile Arg His His Pro Glu
                1475                1480                1485

CAC TTC AGT GGG AGA CCT CGA GAA GAT CGG GTG CCC CAC TCT CGG AAT      4511
His Phe Ser Gly Arg Pro Arg Glu Asp Arg Val Pro His Ser Arg Asn
            1490                1495                1500
```

```
TCC ATC ACC CTC ACC AAC CTC ACT CCA GGC ACA GAG TAT GTG GTC AGC      4559
Ser Ile Thr Leu Thr Asn Leu Thr Pro Gly Thr Glu Tyr Val Val Ser
        1505                1510                1515

ATC GTT GCT CTT AAT GGC AGA GAG GAA AGT CCC TTA TTG ATT GGC CAA      4607
Ile Val Ala Leu Asn Gly Arg Glu Glu Ser Pro Leu Leu Ile Gly Gln
    1520                1525                1530

CAA TCA ACA GTT TCT GAT GTT CCG AGG GAC CTG GAA GTT GTT GCT GCG      4655
Gln Ser Thr Val Ser Asp Val Pro Arg Asp Leu Glu Val Val Ala Ala
1535                1540                1545                1550

ACC CCC ACC AGC CTA CTG ATC AGC TGG GAT GCT CCT GCT GTC ACA GTG      4703
Thr Pro Thr Ser Leu Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val
                1555                1560                1565

AGA TAT TAC AGG ATC ACT TAC GGA GAA ACA GGA GGA AAT AGC CCT GTC      4751
Arg Tyr Tyr Arg Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val
            1570                1575                1580

CAG GAG TTC ACT GTG CCT GGG AGC AAG TCT ACA GCT ACC ATC AGC GGC      4799
Gln Glu Phe Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly
        1585                1590                1595

CTT AAA CCT GGA GTT GAT TAT ACC ATC ACT GTG TAT GCT GTC ACT GGC      4847
Leu Lys Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly
    1600                1605                1610

CGT GGA GAC AGC CCC GCA AGC AGC AAG CCA ATT TCC ATT AAT TAC CGA      4895
Arg Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
1615                1620                1625                1630

ACA GAA ATT GAC AAA CCA TCC CAG ATG CAA GTG ACC GAT GTT CAG GAC      4943
Thr Glu Ile Asp Lys Pro Ser Gln Met Gln Val Thr Asp Val Gln Asp
                1635                1640                1645

AAC AGC ATT AGT GTC AAG TGG CTG CCT TCA AGT TCC CCT GTT ACT GGT      4991
Asn Ser Ile Ser Val Lys Trp Leu Pro Ser Ser Ser Pro Val Thr Gly
            1650                1655                1660

TAC AGA GTA ACC ACC ACT CCC AAA AAT GGA CCA GGA CCA ACA AAA ACT      5039
Tyr Arg Val Thr Thr Thr Pro Lys Asn Gly Pro Gly Pro Thr Lys Thr
        1665                1670                1675

AAA ACT GCA GGT CCA GAT CAA ACA GAA ATG ACT ATT GAA GGC TTG CAG      5087
Lys Thr Ala Gly Pro Asp Gln Thr Glu Met Thr Ile Glu Gly Leu Gln
    1680                1685                1690

CCC ACA GTG GAG TAT GTG GTT AGT GTC TAT GCT CAG AAT CCA AGC GGA      5135
Pro Thr Val Glu Tyr Val Val Ser Val Tyr Ala Gln Asn Pro Ser Gly
1695                1700                1705                1710

GAG AGT CAG CCT CTG GTT CAG ACT GCA GTA ACC AAC ATT GAT CGC CCT      5183
Glu Ser Gln Pro Leu Val Gln Thr Ala Val Thr Asn Ile Asp Arg Pro
                1715                1720                1725
```

```
AAA GGA CTG GCA TTC ACT GAT GTG GAT GTC GAT TCC ATC AAA ATT GCT      5231
Lys Gly Leu Ala Phe Thr Asp Val Asp Val Asp Ser Ile Lys Ile Ala
            1730                1735                1740

TGG GAA AGC CCA CAG GGG CAA GTT TCC AGG TAC AGG GTG ACC TAC TCG      5279
Trp Glu Ser Pro Gln Gly Gln Val Ser Arg Tyr Arg Val Thr Tyr Ser
        1745                1750                1755

AGC CCT GAG GAT GGA ATC CAT GAG CTA TTC CCT GCA CCT GAT GGT GAA      5327
Ser Pro Glu Asp Gly Ile His Glu Leu Phe Pro Ala Pro Asp Gly Glu
    1760                1765                1770

GAA GAC ACT GCA GAG CTG CAA GGC CTC AGA CCG GGT TCT GAG TAC ACA      5375
Glu Asp Thr Ala Glu Leu Gln Gly Leu Arg Pro Gly Ser Glu Tyr Thr
1775                1780                1785                1790

GTC AGT GTG GTT GCC TTG CAC GAT GAT ATG GAG AGC CAG CCC CTG ATT      5423
Val Ser Val Val Ala Leu His Asp Asp Met Glu Ser Gln Pro Leu Ile
                1795                1800                1805

GGA ACC CAG TCC ACA GCT ATT CCT GCA CCA ACT GAC CTG AAG TTC ACT      5471
Gly Thr Gln Ser Thr Ala Ile Pro Ala Pro Thr Asp Leu Lys Phe Thr
            1810                1815                1820

CAG GTC ACA CCC ACA AGC CTG AGC GCC CAG TGG ACA CCA CCC AAT GTT      5519
Gln Val Thr Pro Thr Ser Leu Ser Ala Gln Trp Thr Pro Pro Asn Val
        1825                1830                1835

CAG CTC ACT GGA TAT CGA GTG CGG GTG ACC CCC AAG GAG AAG ACC GGA      5567
Gln Leu Thr Gly Tyr Arg Val Arg Val Thr Pro Lys Glu Lys Thr Gly
    1840                1845                1850

CCA ATG AAA GAA ATC AAC CTT GCT CCT GAC AGC TCA TCC GTG GTT GTA      5615
Pro Met Lys Glu Ile Asn Leu Ala Pro Asp Ser Ser Ser Val Val Val
1855                1860                1865                1870

TCA GGA CTT ATG GTG GCC ACC AAA TAT GAA GTG AGT GTC TAT GCT CTT      5663
Ser Gly Leu Met Val Ala Thr Lys Tyr Glu Val Ser Val Tyr Ala Leu
                1875                1880                1885

AAG GAC ACT TTG ACA AGC AGA CCA GCT CAG GGT GTT GTC ACC ACT CTG      5711
Lys Asp Thr Leu Thr Ser Arg Pro Ala Gln Gly Val Val Thr Thr Leu
            1890                1895                1900

GAG AAT GTC AGC CCA CCA AGA AGG GCT CGT GTG ACA GAT GCT ACT GAG      5759
Glu Asn Val Ser Pro Pro Arg Arg Ala Arg Val Thr Asp Ala Thr Glu
        1905                1910                1915

ACC ACC ATC ACC ATT AGC TGG AGA ACC AAG ACT GAG ACG ATC ACT GGC      5807
Thr Thr Ile Thr Ile Ser Trp Arg Thr Lys Thr Glu Thr Ile Thr Gly
    1920                1925                1930
```

```
TTC CAA GTT GAT GCC GTT CCA GCC AAT GGC CAG ACT CCA ATC CAG AGA      5855
Phe Gln Val Asp Ala Val Pro Ala Asn Gly Gln Thr Pro Ile Gln Arg
1935                1940                1945                1950

ACC ATC AAG CCA GAT GTC AGA AGC TAC ACC ATC ACA GGT TTA CAA CCA      5903
Thr Ile Lys Pro Asp Val Arg Ser Tyr Thr Ile Thr Gly Leu Gln Pro
                1955                1960                1965

GGC ACT GAC TAC AAG ATC TAC CTG TAC ACC TTG AAT GAC AAT GCT CGG      5951
Gly Thr Asp Tyr Lys Ile Tyr Leu Tyr Thr Leu Asn Asp Asn Ala Arg
            1970                1975                1980

AGC TCC CCT GTG GTC ATC GAC GCC TCC ACT GCC ATT GAT GCA CCA TCC      5999
Ser Ser Pro Val Val Ile Asp Ala Ser Thr Ala Ile Asp Ala Pro Ser
        1985                1990                1995

AAC CTG CGT TTC CTG GCC ACC ACA CCC AAT TCC TTG CTG GTA TCA TGG      6047
Asn Leu Arg Phe Leu Ala Thr Thr Pro Asn Ser Leu Leu Val Ser Trp
    2000                2005                2010

CAG CCG CCA CGT GCC AGG ATT ACC GGC TAC ATC ATC AAG TAT GAG AAG      6095
Gln Pro Pro Arg Ala Arg Ile Thr Gly Tyr Ile Ile Lys Tyr Glu Lys
2015                2020                2025                2030

CCT GGG TCT CCT CCC AGA GAA GTG GTC CCT CGG CCC CGC CCT GGT GTC      6143
Pro Gly Ser Pro Pro Arg Glu Val Val Pro Arg Pro Arg Pro Gly Val
                2035                2040                2045

ACA GAG GCT ACT ATT ACT GGC CTG GAA CCG GGA ACC GAA TAT ACA ATT      6191
Thr Glu Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr Glu Tyr Thr Ile
            2050                2055                2060

TAT GTC ATT GCC CTG AAG AAT AAT CAG AAG AGC GAG CCC CTG ATT GGA      6239
Tyr Val Ile Ala Leu Lys Asn Asn Gln Lys Ser Glu Pro Leu Ile Gly
        2065                2070                2075

AGG AAA AAG ACA GAC GAG CTT CCC CAA CTG GTA ACC CTT CCA CAC CCC      6287
Arg Lys Lys Thr Asp Glu Leu Pro Gln Leu Val Thr Leu Pro His Pro
    2080                2085                2090

AAT CTT CAT GGA CCA GAG ATC TTG GAT GTT CCT TCC ACA GTT CAA AAG      6335
Asn Leu His Gly Pro Glu Ile Leu Asp Val Pro Ser Thr Val Gln Lys
2095                2100                2105                2110

ACC CCT TTC GTC ACC CAC CCT GGG TAT GAC ACT GGA AAT GGT ATT CAG      6383
Thr Pro Phe Val Thr His Pro Gly Tyr Asp Thr Gly Asn Gly Ile Gln
                2115                2120                2125

CTT CCT GGC ACT TCT GGT CAG CAA CCC AGT GTT GGG CAA CAA ATG ATC      6431
Leu Pro Gly Thr Ser Gly Gln Gln Pro Ser Val Gly Gln Gln Met Ile
            2130                2135                2140

TTT GAG GAA CAT GGT TTT AGG CGG ACC ACA CCG CCC ACA ACG GCC ACC      6479
Phe Glu Glu His Gly Phe Arg Arg Thr Thr Pro Pro Thr Thr Ala Thr
        2145                2150                2155
```

```
CCC ATA AGG CAT AGG CCA AGA CCA TAC CCG CCG AAT GTA GGA CAA GAA      6527
Pro Ile Arg His Arg Pro Arg Pro Tyr Pro Pro Asn Val Gly Gln Glu
    2160                2165                2170

GCT CTC TCT CAG ACA ACC ATC TCA TGG GCC CCA TTC CAG GAC ACT TCT      6575
Ala Leu Ser Gln Thr Thr Ile Ser Trp Ala Pro Phe Gln Asp Thr Ser
2175                2180                2185                2190

GAG TAC ATC ATT TCA TGT CAT CCT GTT GGC ACT GAT GAA GAA CCC TTA      6623
Glu Tyr Ile Ile Ser Cys His Pro Val Gly Thr Asp Glu Glu Pro Leu
                2195                2200                2205

CAG TTC AGG GTT CCT GGA ACT TCT ACC AGT GCC ACT CTG ACA GGC CTC      6671
Gln Phe Arg Val Pro Gly Thr Ser Thr Ser Ala Thr Leu Thr Gly Leu
            2210                2215                2220

ACC AGA GGT GCC ACC TAC AAC ATC ATA GTG GAG GCA CTG AAA GAC CAG      6719
Thr Arg Gly Ala Thr Tyr Asn Ile Ile Val Glu Ala Leu Lys Asp Gln
        2225                2230                2235

CAG AGG CAT AAG GTT CGG GAA GAG GTT GTT ACC GTG GGC AAC TCT GTC      6767
Gln Arg His Lys Val Arg Glu Glu Val Val Thr Val Gly Asn Ser Val
    2240                2245                2250

AAC GAA GGC TTG AAC CAA CCT ACG GAT GAC TCG TGC TTT GAC CCC TAC      6815
Asn Glu Gly Leu Asn Gln Pro Thr Asp Asp Ser Cys Phe Asp Pro Tyr
2255                2260                2265                2270

ACA GTT TCC CAT TAT GCC GTT GGA GAT GAG TGG GAA CGA ATG TCT GAA      6863
Thr Val Ser His Tyr Ala Val Gly Asp Glu Trp Glu Arg Met Ser Glu
                2275                2280                2285

TCA GGC TTT AAA CTG TTG TGC CAG TGC TTA GGC TTT GGA AGT GGT CAT      6911
Ser Gly Phe Lys Leu Leu Cys Gln Cys Leu Gly Phe Gly Ser Gly His
            2290                2295                2300

TTC AGA TGT GAT TCA TCT AGA TGG TGC CAT GAC AAT GGT GTG AAC TAC      6959
Phe Arg Cys Asp Ser Ser Arg Trp Cys His Asp Asn Gly Val Asn Tyr
        2305                2310                2315

AAG ATT GGA GAG AAG TGG GAC CGT CAG GGA GAA AAT GGC CAG ATG ATG      7007
Lys Ile Gly Glu Lys Trp Asp Arg Gln Gly Glu Asn Gly Gln Met Met
    2320                2325                2330

AGC TGC ACA TGT CTT GGG AAC GGA AAA GGA GAA TTC AAG TGT GAC CCT      7055
Ser Cys Thr Cys Leu Gly Asn Gly Lys Gly Glu Phe Lys Cys Asp Pro
2335                2340                2345                2350

CAT GAG GCA ACG TGT TAC GAT GAT GGG AAG ACA TAC CAC GTA GGA GAA      7103
His Glu Ala Thr Cys Tyr Asp Asp Gly Lys Thr Tyr His Val Gly Glu
                2355                2360                2365
```

```
CAG TGG CAG AAG GAA TAT CTC GGT GCC ATT TGC TCC TGC ACA TGC TTT      7151
Gln Trp Gln Lys Glu Tyr Leu Gly Ala Ile Cys Ser Cys Thr Cys Phe
          2370                2375                2380

GGA GGC CAG CGG GGC TGG CGC TGT GAC AAC TGC CGC AGA CCT GGG GGT      7199
Gly Gly Gln Arg Gly Trp Arg Cys Asp Asn Cys Arg Arg Pro Gly Gly
      2385                2390                2395

GAA CCC AGT CCC GAA GGC ACT ACT GGC CAG TCC TAC AAC CAG TAT TCT      7247
Glu Pro Ser Pro Glu Gly Thr Thr Gly Gln Ser Tyr Asn Gln Tyr Ser
  2400                2405                2410

CAG AGA TAC CAT CAG AGA ACA AAC ACT AAT GTT AAT TGC CCA ATT GAG      7295
Gln Arg Tyr His Gln Arg Thr Asn Thr Asn Val Asn Cys Pro Ile Glu
2415                2420                2425                2430

TGC TTC ATG CCT TTA GAT GTA CAG GCT GAC AGA GAA GAT TCC CGA GAG      7343
Cys Phe Met Pro Leu Asp Val Gln Ala Asp Arg Glu Asp Ser Arg Glu
                2435                2440                2445

TAAATCATCT TTCCAATCCA GAGGAACAAG CATGTCTCTC TGCCAAGATC CATCTAAACT    7403

GGAGTGATGT TAGCAGACCC AGCTTAGAGT TCTTCTTTCT TTCTTAAGCC CTTTGCTCTG    7463

GAGGAAGTTC TCCAGCTTCA GCTCAACTCA CAGCTTCTCC AAGCATCACC CTGGGAGTTT    7523

CCTGAGGGTT TTCTCATAAA TGAGGGCTGC ACATTGCCTG TTCTGCTTCG AAGTATTCAA    7583

TACCGCTCAG TATTTTAAAT GAAGTGATTC TAAGATTTGG TTTGGGATCA ATAGGAAAGC    7643

ATATGCAGCC AACCAAGATG CAAATGTTTT GAAATGATAT GACCAAAATT TTAAGTAGGA    7703

AAGTCACCCA AACACTTCTG CTTTCACTTA AGTGTCTGGC CCGCAATACT GTAGGAACAA    7763

GCATGATCTT GTTACTGTGA TATTTTAAAT ATCCACAGTA                          7803
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2446 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Leu Arg Gly Pro Gly Pro Gly Leu Leu Leu Leu Ala Val Leu Cys
 1               5                  10                  15

Leu Gly Thr Ala Val Pro Ser Thr Gly Ala Ser Lys Ser Lys Arg Gln
            20                  25                  30
```

```
Ala Gln Gln Met Val Gln Pro Gln Ser Pro Val Ala Val Ser Gln Ser
        35                  40                  45

Lys Pro Gly Cys Tyr Asp Asn Gly Lys His Tyr Gln Ile Asn Gln Gln
    50                  55                  60

Trp Glu Arg Thr Tyr Leu Gly Asn Val Leu Val Cys Thr Cys Tyr Gly
65                  70                  75                  80

Gly Ser Arg Gly Phe Asn Cys Glu Ser Lys Pro Glu Ala Glu Glu Thr
                85                  90                  95

Cys Phe Asp Lys Tyr Thr Gly Asn Thr Tyr Arg Val Gly Asp Thr Tyr
            100                 105                 110

Glu Arg Pro Lys Asp Ser Met Ile Trp Asp Cys Thr Cys Ile Gly Ala
        115                 120                 125

Gly Arg Gly Arg Ile Ser Cys Thr Ile Ala Asn Arg Cys His Glu Gly
    130                 135                 140

Gly Gln Ser Tyr Lys Ile Gly Asp Thr Trp Arg Arg Pro His Glu Thr
145                 150                 155                 160

Gly Gly Tyr Met Leu Glu Cys Val Cys Leu Gly Asn Gly Lys Gly Glu
                165                 170                 175

Trp Thr Cys Lys Pro Ile Ala Glu Lys Cys Phe Asp His Ala Ala Gly
            180                 185                 190

Thr Ser Tyr Val Val Gly Glu Thr Trp Glu Lys Pro Tyr Gln Gly Trp
        195                 200                 205

Met Met Val Asp Cys Thr Cys Leu Gly Glu Gly Ser Gly Arg Ile Thr
    210                 215                 220

Cys Thr Ser Arg Asn Arg Cys Asn Asp Gln Asp Thr Arg Thr Ser Tyr
225                 230                 235                 240

Arg Ile Gly Asp Thr Trp Ser Lys Lys Asp Asn Arg Gly Asn Leu Leu
                245                 250                 255

Gln Cys Ile Cys Thr Gly Asn Gly Arg Gly Glu Trp Lys Cys Glu Arg
            260                 265                 270

His Thr Ser Val Gln Thr Thr Ser Ser Gly Ser Gly Pro Phe Thr Asp
        275                 280                 285

Val Arg Ala Ala Val Tyr Gln Pro Gln Pro His Pro Gln Pro Pro Pro
    290                 295                 300

Tyr Gly His Cys Val Thr Asp Ser Gly Val Val Tyr Ser Val Gly Met
305                 310                 315                 320
```

Gln Trp Leu Lys Thr Gln Gly Asn Lys Gln Met Leu Cys Thr Cys Leu
325 330 335

Gly Asn Gly Val Ser Cys Gln Glu Thr Ala Val Thr Gln Thr Tyr Gly
340 345 350

Gly Asn Leu Asn Gly Glu Pro Cys Val Leu Pro Phe Thr Tyr Asn Gly
355 360 365

Arg Thr Phe Tyr Ser Cys Thr Thr Glu Gly Arg Gln Asp Gly His Leu
370 375 380

Trp Cys Ser Thr Thr Ser Asn Tyr Glu Gln Asp Gln Lys Tyr Ser Phe
385 390 395 400

Cys Thr Asp His Thr Val Leu Val Gln Thr Gln Gly Gly Asn Ser Asn
405 410 415

Gly Ala Leu Cys His Phe Pro Phe Leu Tyr Asn Asn His Asn Tyr Thr
420 425 430

Asp Cys Thr Ser Glu Gly Arg Arg Asp Asn Met Lys Trp Cys Gly Thr
435 440 445

Thr Gln Asn Tyr Asp Ala Asp Gln Lys Phe Gly Phe Cys Pro Met Ala
450 455 460

Ala His Glu Glu Ile Cys Thr Thr Asn Glu Gly Val Met Tyr Arg Ile
465 470 475 480

Gly Asp Gln Trp Asp Lys Gln His Asp Met Gly His Met Met Arg Cys
485 490 495

Thr Cys Val Gly Asn Gly Arg Gly Glu Trp Thr Cys Ile Ala Tyr Ser
500 505 510

Gln Leu Arg Asp Gln Cys Ile Val Asp Asp Ile Thr Tyr Asn Val Asn
515 520 525

Asp Thr Phe His Lys Arg His Glu Glu Gly His Met Leu Asn Cys Thr
530 535 540

Cys Phe Gly Gln Gly Arg Gly Arg Trp Lys Cys Asp Pro Val Asp Gln
545 550 555 560

Cys Gln Asp Ser Glu Thr Gly Thr Phe Tyr Gln Ile Gly Asp Ser Trp
565 570 575

Glu Lys Tyr Val His Gly Val Arg Tyr Gln Cys Tyr Cys Tyr Gly Arg
580 585 590

Gly Ile Gly Glu Trp His Cys Gln Pro Leu Gln Thr Tyr Pro Ser Ser
595 600 605

Ser Gly Pro Val Glu Val Phe Ile Thr Glu Thr Pro Ser Gln Pro Asn
610 615 620

Ser His Pro Ile Gln Trp Asn Ala Pro Gln Pro Ser His Ile Ser Lys
625 630 635 640

Tyr Ile Leu Arg Trp Arg Pro Lys Asn Ser Val Gly Arg Trp Lys Glu
645 650 655

Ala Thr Ile Pro Gly His Leu Asn Ser Tyr Thr Ile Lys Gly Leu Lys
660 665 670

Pro Gly Val Val Tyr Glu Gly Gln Leu Ile Ser Ile Gln Gln Tyr Gly
675 680 685

His Gln Glu Val Thr Arg Phe Asp Phe Thr Thr Thr Ser Thr Ser Thr
690 695 700

Pro Val Thr Ser Asn Thr Val Thr Gly Glu Thr Thr Pro Phe Ser Pro
705 710 715 720

Leu Val Ala Thr Ser Glu Ser Val Thr Glu Ile Thr Ala Ser Ser Phe
725 730 735

Val Val Ser Trp Val Ser Ala Ser Asp Thr Val Ser Gly Phe Arg Val
740 745 750

Glu Tyr Glu Leu Ser Glu Glu Gly Asp Glu Pro Gln Tyr Leu Asp Leu
755 760 765

Pro Ser Thr Ala Thr Ser Val Asn Ile Pro Asp Leu Leu Pro Gly Arg
770 775 780

Lys Tyr Ile Val Asn Val Tyr Gln Ile Ser Glu Asp Gly Glu Gln Ser
785 790 795 800

Leu Ile Leu Ser Thr Ser Gln Thr Thr Ala Pro Asp Ala Pro Pro Asp
805 810 815

Pro Thr Val Asp Gln Val Asp Asp Thr Ser Ile Val Val Arg Trp Ser
820 825 830

Arg Pro Gln Ala Pro Ile Thr Gly Tyr Arg Ile Val Tyr Ser Pro Ser
835 840 845

Val Glu Gly Ser Ser Thr Glu Leu Asn Leu Pro Glu Thr Ala Asn Ser
850 855 860

Val Thr Leu Ser Asp Leu Gln Pro Gly Val Gln Tyr Asn Ile Thr Ile
865 870 875 880

Tyr Ala Val Glu Glu Asn Gln Glu Ser Thr Pro Val Val Ile Gln Gln
885 890 895

Glu Thr Thr Gly Thr Pro Arg Ser Asp Thr Val Pro Ser Pro Arg Asp
900 905 910

Leu Gln Phe Val Glu Val Thr Asp Val Lys Val Thr Ile Met Trp Thr
915 920 925

Pro Pro Glu Ser Ala Val Thr Gly Tyr Arg Val Asp Val Ile Pro Val
930 935 940

Asn Leu Pro Gly Glu His Gly Gln Arg Leu Pro Ile Ser Arg Asn Thr
945 950 955 960

Phe Ala Glu Val Thr Gly Leu Ser Pro Gly Val Thr Tyr Tyr Phe Lys
965 970 975

Val Phe Ala Val Ser His Gly Arg Glu Ser Lys Pro Leu Thr Ala Gln
980 985 990

Gln Thr Thr Lys Leu Asp Ala Pro Thr Asn Leu Gln Phe Val Asn Glu
995 1000 1005

Thr Asp Ser Thr Val Leu Val Arg Trp Thr Pro Pro Arg Ala Gln Ile
1010 1015 1020

Thr Gly Tyr Arg Leu Thr Val Gly Leu Thr Arg Arg Gly Gln Pro Arg
1025 1030 1035 1040

Gln Tyr Asn Val Gly Pro Ser Val Ser Lys Tyr Pro Leu Arg Asn Leu
1045 1050 1055

Gln Pro Ala Ser Glu Tyr Thr Val Ser Leu Val Ala Ile Lys Gly Asn
1060 1065 1070

Gln Glu Ser Pro Lys Ala Thr Gly Val Phe Thr Thr Leu Gln Pro Gly
1075 1080 1085

Ser Ser Ile Pro Pro Tyr Asn Thr Glu Val Thr Glu Thr Thr Ile Val
1090 1095 1100

Ile Thr Trp Thr Pro Ala Pro Arg Ile Gly Phe Lys Leu Gly Val Arg
1105 1110 1115 1120

Pro Ser Gln Gly Gly Glu Ala Pro Arg Glu Val Thr Ser Asp Ser Gly
1125 1130 1135

Ser Ile Val Val Ser Gly Leu Thr Pro Gly Val Glu Tyr Val Tyr Thr
1140 1145 1150

Ile Gln Val Leu Arg Asp Gly Gln Glu Arg Asp Ala Pro Ile Val Asn
1155 1160 1165

Lys Val Val Thr Pro Leu Ser Pro Pro Thr Asn Leu His Leu Glu Ala
1170 1175 1180

Asn Pro Asp Thr Gly Val Leu Thr Val Ser Trp Glu Arg Ser Thr Thr
1185 1190 1195 1200

Pro Asp Ile Thr Gly Tyr Arg Ile Thr Thr Thr Pro Thr Asn Gly Gln
1205 1210 1215

Gln Gly Asn Ser Leu Glu Glu Val Val His Ala Asp Gln Ser Ser Cys
1220 1225 1230

Thr Phe Asp Asn Leu Ser Pro Gly Leu Glu Tyr Asn Val Ser Val Tyr
1235 1240 1245

Thr Val Lys Asp Asp Lys Glu Ser Val Pro Ile Ser Asp Thr Ile Ile
1250 1255 1260

Pro Glu Val Pro Gln Leu Thr Asp Leu Ser Phe Val Asp Ile Thr Asp
1265 1270 1275 1280

Ser Ser Ile Gly Leu Arg Trp Thr Pro Leu Asn Ser Ser Thr Ile Ile
1285 1290 1295

Gly Tyr Arg Ile Thr Val Val Ala Ala Gly Glu Gly Ile Pro Ile Phe
1300 1305 1310

Glu Asp Phe Val Tyr Ser Ser Val Gly Tyr Tyr Thr Val Thr Gly Leu
1315 1320 1325

Glu Pro Gly Ile Asp Tyr Asp Ile Ser Val Ile Thr Leu Ile Asn Gly
1330 1335 1340

Gly Glu Ser Ala Pro Thr Thr Leu Thr Gln Gln Thr Ala Val Pro Pro
1345 1350 1355 1360

Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg Val
1365 1370 1375

Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu Val Arg
1380 1385 1390

Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu Ser Ile Ser
1395 1400 1405

Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu Pro Gly Thr Glu
1410 1415 1420

Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln His Glu Ser Thr Pro
1425 1430 1435 1440

Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp Ser Pro Thr Gly Ile Asp
1445 1450 1455

Phe Ser Asp Ile Thr Ala Asn Ser Phe Thr Val His Trp Ile Ala Pro
1460 1465 1470

Arg Ala Thr Ile Thr Gly Tyr Arg Ile Arg His His Pro Glu His Phe
1475 1480 1485

Ser Gly Arg Pro Arg Glu Asp Arg Val Pro His Ser Arg Asn Ser Ile
1490 1495 1500

Thr Leu Thr Asn Leu Thr Pro Gly Thr Glu Tyr Val Val Ser Ile Val
1505 1510 1515 1520

Ala Leu Asn Gly Arg Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser
1525 1530 1535

Thr Val Ser Asp Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro
1540 1545 1550

Thr Ser Leu Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr
1555 1560 1565

Tyr Arg Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu
1570 1575 1580

Phe Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
1585 1590 1595 1600

Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg Gly
1605 1610 1615

Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg Thr Glu
1620 1625 1630

Ile Asp Lys Pro Ser Gln Met Gln Val Thr Asp Val Gln Asp Asn Ser
1635 1640 1645

Ile Ser Val Lys Trp Leu Pro Ser Ser Ser Pro Val Thr Gly Tyr Arg
1650 1655 1660

Val Thr Thr Thr Pro Lys Asn Gly Pro Gly Pro Thr Lys Thr Lys Thr
1665 1670 1675 1680

Ala Gly Pro Asp Gln Thr Glu Met Thr Ile Glu Gly Leu Gln Pro Thr
1685 1690 1695

Val Glu Tyr Val Val Ser Val Tyr Ala Gln Asn Pro Ser Gly Glu Ser
1700 1705 1710

Gln Pro Leu Val Gln Thr Ala Val Thr Asn Ile Asp Arg Pro Lys Gly
1715 1720 1725

Leu Ala Phe Thr Asp Val Asp Val Asp Ser Ile Lys Ile Ala Trp Glu
1730 1735 1740

Ser Pro Gln Gly Gln Val Ser Arg Tyr Arg Val Thr Tyr Ser Ser Pro
1745 1750 1755 1760

Glu Asp Gly Ile His Glu Leu Phe Pro Ala Pro Asp Gly Glu Glu Asp
1765 1770 1775

Thr Ala Glu Leu Gln Gly Leu Arg Pro Gly Ser Glu Tyr Thr Val Ser
1780 1785 1790

Val Val Ala Leu His Asp Asp Met Glu Ser Gln Pro Leu Ile Gly Thr
1795 1800 1805

Gln Ser Thr Ala Ile Pro Ala Pro Thr Asp Leu Lys Phe Thr Gln Val
1810 1815 1820

Thr Pro Thr Ser Leu Ser Ala Gln Trp Thr Pro Pro Asn Val Gln Leu
1825 1830 1835 1840

Thr Gly Tyr Arg Val Arg Val Thr Pro Lys Glu Lys Thr Gly Pro Met
1845 1850 1855

Lys Glu Ile Asn Leu Ala Pro Asp Ser Ser Ser Val Val Val Ser Gly
1860 1865 1870

Leu Met Val Ala Thr Lys Tyr Glu Val Ser Val Tyr Ala Leu Lys Asp
1875 1880 1885

Thr Leu Thr Ser Arg Pro Ala Gln Gly Val Val Thr Thr Leu Glu Asn
1890 1895 1900

Val Ser Pro Pro Arg Arg Ala Arg Val Thr Asp Ala Thr Glu Thr Thr
1905 1910 1915 1920

Ile Thr Ile Ser Trp Arg Thr Lys Thr Glu Thr Ile Thr Gly Phe Gln
1925 1930 1935

Val Asp Ala Val Pro Ala Asn Gly Gln Thr Pro Ile Gln Arg Thr Ile
1940 1945 1950

Lys Pro Asp Val Arg Ser Tyr Thr Ile Thr Gly Leu Gln Pro Gly Thr
1955 1960 1965

Asp Tyr Lys Ile Tyr Leu Tyr Thr Leu Asn Asp Asn Ala Arg Ser Ser
1970 1975 1980

Pro Val Val Ile Asp Ala Ser Thr Ala Ile Asp Ala Pro Ser Asn Leu
1985 1990 1995 2000

Arg Phe Leu Ala Thr Thr Pro Asn Ser Leu Leu Val Ser Trp Gln Pro
2005 2010 2015

Pro Arg Ala Arg Ile Thr Gly Tyr Ile Ile Lys Tyr Glu Lys Pro Gly
2020 2025 2030

Ser Pro Pro Arg Glu Val Val Pro Arg Pro Arg Pro Gly Val Thr Glu
2035 2040 2045

Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr Glu Tyr Thr Ile Tyr Val
2050 2055 2060

Ile Ala Leu Lys Asn Asn Gln Lys Ser Glu Pro Leu Ile Gly Arg Lys
2065 2070 2075 2080

Lys Thr Asp Glu Leu Pro Gln Leu Val Thr Leu Pro His Pro Asn Leu
2085 2090 2095

His Gly Pro Glu Ile Leu Asp Val Pro Ser Thr Val Gln Lys Thr Pro
2100 2105 2110

Phe Val Thr His Pro Gly Tyr Asp Thr Gly Asn Gly Ile Gln Leu Pro
2115 2120 2125

Gly Thr Ser Gly Gln Gln Pro Ser Val Gly Gln Gln Met Ile Phe Glu
2130 2135 2140

Glu His Gly Phe Arg Arg Thr Thr Pro Pro Thr Thr Ala Thr Pro Ile
2145 2150 2155 2160

Arg His Arg Pro Arg Pro Tyr Pro Pro Asn Val Gly Gln Glu Ala Leu
2165 2170 2175

Ser Gln Thr Thr Ile Ser Trp Ala Pro Phe Gln Asp Thr Ser Glu Tyr
2180 2185 2190

Ile Ile Ser Cys His Pro Val Gly Thr Asp Glu Glu Pro Leu Gln Phe
2195 2200 2205

Arg Val Pro Gly Thr Ser Thr Ser Ala Thr Leu Thr Gly Leu Thr Arg
2210 2215 2220

Gly Ala Thr Tyr Asn Ile Ile Val Glu Ala Leu Lys Asp Gln Gln Arg
2225 2230 2235 2240

His Lys Val Arg Glu Glu Val Val Thr Val Gly Asn Ser Val Asn Glu
2245 2250 2255

Gly Leu Asn Gln Pro Thr Asp Asp Ser Cys Phe Asp Pro Tyr Thr Val
2260 2265 2270

Ser His Tyr Ala Val Gly Asp Glu Trp Glu Arg Met Ser Glu Ser Gly
2275 2280 2285

Phe Lys Leu Leu Cys Gln Cys Leu Gly Phe Gly Ser Gly His Phe Arg
2290 2295 2300

Cys Asp Ser Ser Arg Trp Cys His Asp Asn Gly Val Asn Tyr Lys Ile
2305 2310 2315 2320

Gly Glu Lys Trp Asp Arg Gln Gly Glu Asn Gly Gln Met Met Ser Cys
2325 2330 2335

```
Thr Cys Leu Gly Asn Gly Lys Gly Glu Phe Lys Cys Asp Pro His Glu
            2340                2345                2350

Ala Thr Cys Tyr Asp Asp Gly Lys Thr Tyr His Val Gly Glu Gln Trp
        2355                2360                2365

Gln Lys Glu Tyr Leu Gly Ala Ile Cys Ser Cys Thr Cys Phe Gly Gly
    2370                2375                2380

Gln Arg Gly Trp Arg Cys Asp Asn Cys Arg Arg Pro Gly Gly Glu Pro
2385                2390                2395                2400

Ser Pro Glu Gly Thr Thr Gly Gln Ser Tyr Asn Gln Tyr Ser Gln Arg
                2405                2410                2415

Tyr His Gln Arg Thr Asn Thr Asn Val Asn Cys Pro Ile Glu Cys Phe
            2420                2425                2430

Met Pro Leu Asp Val Gln Ala Asp Arg Glu Asp Ser Arg Glu
        2435                2440                2445
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2179 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 31..1962

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
GTCTAGGAGC CAGCCCCACC CTTAGAAAAG ATG TTT TCC ATG AGG ATC GTC TGC      54
                                 Met Phe Ser Met Arg Ile Val Cys
                                   1               5

CTA GTT CTA AGT GTG GTG GGC ACA GCA TGG ACT GCA GAT AGT GGT GAA      102
Leu Val Leu Ser Val Val Gly Thr Ala Trp Thr Ala Asp Ser Gly Glu
 10                  15                  20

GGT GAC TTT CTA GCT GAA GGA GGA GGC GTG CGT GGC CCA AGG GTT GTG      150
Gly Asp Phe Leu Ala Glu Gly Gly Gly Val Arg Gly Pro Arg Val Val
25                  30                  35                  40

GAA AGA CAT CAA TCT GCC TGC AAA GAT TCA GAC TGG CCC TTC TGC TCT      198
Glu Arg His Gln Ser Ala Cys Lys Asp Ser Asp Trp Pro Phe Cys Ser
                45                  50                  55
```

```
GAT GAA GAC TGG AAC TAC AAA TGC CCT TCT GGC TGC AGG ATG AAA GGG      246
Asp Glu Asp Trp Asn Tyr Lys Cys Pro Ser Gly Cys Arg Met Lys Gly
            60                  65                  70

TTG ATT GAT GAA GTC AAT CAA GAT TTT ACA AAC AGA ATA AAT AAG CTC      294
Leu Ile Asp Glu Val Asn Gln Asp Phe Thr Asn Arg Ile Asn Lys Leu
        75                  80                  85

AAA AAT TCA CTA TTT GAA TAT CAG AAG AAC AAT AAG GAT TCT CAT TCG      342
Lys Asn Ser Leu Phe Glu Tyr Gln Lys Asn Asn Lys Asp Ser His Ser
    90                  95                  100

TTG ACC ACT AAT ATA ATG GAA ATT TTG AGA GGC GAT TTT TCC TCA GCC      390
Leu Thr Thr Asn Ile Met Glu Ile Leu Arg Gly Asp Phe Ser Ser Ala
105                 110                 115                 120

AAT AAC CGT GAT AAT ACC TAC AAC CGA GTG TCA GAG GAT CTG AGA AGC      438
Asn Asn Arg Asp Asn Thr Tyr Asn Arg Val Ser Glu Asp Leu Arg Ser
                125                 130                 135

AGA ATT GAA GTC CTG AAG CGC AAA GTC ATA GAA AAA GTA CAG CAT ATC      486
Arg Ile Glu Val Leu Lys Arg Lys Val Ile Glu Lys Val Gln His Ile
            140                 145                 150

CAG CTT CTG CAG AAA AAT GTT AGA GCT CAG TTG GTT GAT ATG AAA CGA      534
Gln Leu Leu Gln Lys Asn Val Arg Ala Gln Leu Val Asp Met Lys Arg
        155                 160                 165

CTG GAG GTG GAC ATT GAT ATT AAG ATC CGA TCT TGT CGA GGG TCA TGG      582
Leu Glu Val Asp Ile Asp Ile Lys Ile Arg Ser Cys Arg Gly Ser Trp
    170                 175                 180

AGT AGG GCT TTA GCT CGT GAA GTA GAT CTG AAG GAC TAT GAA GAT CAG      630
Ser Arg Ala Leu Ala Arg Glu Val Asp Leu Lys Asp Tyr Glu Asp Gln
185                 190                 195                 200

CAG AAG CAA CTT GAA CAG GTC ATT GCC AAA GAC TTA CTT CCC TCT AGA      678
Gln Lys Gln Leu Glu Gln Val Ile Ala Lys Asp Leu Leu Pro Ser Arg
                205                 210                 215

GAT AGG CAA CAC TTA CCA CTG ATA AAA ATG AAA CCA GTT CCA GAC TTG      726
Asp Arg Gln His Leu Pro Leu Ile Lys Met Lys Pro Val Pro Asp Leu
            220                 225                 230

GTT CCC GGA AAT TTT AAG AGC CAG CTT CAG AAG GTA CCC CCA GAG TGG      774
Val Pro Gly Asn Phe Lys Ser Gln Leu Gln Lys Val Pro Pro Glu Trp
        235                 240                 245

AAG GCA TTA ACA GAC ATG CCG CAG ATG AGA ATG GAG TTA GAG AGA CCT      822
Lys Ala Leu Thr Asp Met Pro Gln Met Arg Met Glu Leu Glu Arg Pro
    250                 255                 260

GGT GGA AAT GAG ATT ACT CGA GGA GGC TCC ACC TCT TAT GGA ACC GGA      870
Gly Gly Asn Glu Ile Thr Arg Gly Gly Ser Thr Ser Tyr Gly Thr Gly
265                 270                 275                 280
```

```
TCA GAG ACG GAA AGC CCC AGG AAC CCT AGC AGT GCT GGA AGC TGG AAC      918
Ser Glu Thr Glu Ser Pro Arg Asn Pro Ser Ser Ala Gly Ser Trp Asn
                285                 290                 295

TCT GGG AGC TCT GGA CCT GGA AGT ACT GGA AAC CGA AAC CCT GGG AGC      966
Ser Gly Ser Ser Gly Pro Gly Ser Thr Gly Asn Arg Asn Pro Gly Ser
            300                 305                 310

TCT GGG ACT GGA GGG ACT GCA ACC TGG AAA CCT GGG AGC TCT GGA CCT     1014
Ser Gly Thr Gly Gly Thr Ala Thr Trp Lys Pro Gly Ser Ser Gly Pro
        315                 320                 325

GGA AGT GCT GGA AGC TGG AAC TCT GGG AGC TCT GGA ACT GGA AGT ACT     1062
Gly Ser Ala Gly Ser Trp Asn Ser Gly Ser Ser Gly Thr Gly Ser Thr
    330                 335                 340

GGA AAC CAA AAC CCT GGA AGT CCT AGA CCT GGT AGT ACC GGA ACC TGG     1110
Gly Asn Gln Asn Pro Gly Ser Pro Arg Pro Gly Ser Thr Gly Thr Trp
345                 350                 355                 360

AAT CCT GGC AGC TCT GAA CGC GGA AGT GCT GGG CAC TGG ACC TCT GAG     1158
Asn Pro Gly Ser Ser Glu Arg Gly Ser Ala Gly His Trp Thr Ser Glu
                365                 370                 375

AGC TCT GTA TCT GGT AGT ACT GGA CAA TGG CAC TCT GAA TCT GGA AGT     1206
Ser Ser Val Ser Gly Ser Thr Gly Gln Trp His Ser Glu Ser Gly Ser
            380                 385                 390

TTT AGG CCA GAT AGC CCA GGC TCT GGG AAC GCG AGG CCT AAC AAC CCA     1254
Phe Arg Pro Asp Ser Pro Gly Ser Gly Asn Ala Arg Pro Asn Asn Pro
        395                 400                 405

GAC TGG GGC ACA TTT GAA GAG GTG TCA GGA AAT GTA AGT CCA GGG ACA     1302
Asp Trp Gly Thr Phe Glu Glu Val Ser Gly Asn Val Ser Pro Gly Thr
    410                 415                 420

AGG AGA GAG TAC CAC ACA GAA AAA CTG GTC ACT AAA GGA GAT AAA GAG     1350
Arg Arg Glu Tyr His Thr Glu Lys Leu Val Thr Lys Gly Asp Lys Glu
425                 430                 435                 440

CTC AGG ACT GGT AAA GAG AAG GTC ACC TCT GGT AGC ACA ACC ACC ACG     1398
Leu Arg Thr Gly Lys Glu Lys Val Thr Ser Gly Ser Thr Thr Thr Thr
                445                 450                 455

CGT CGT TCA TGC TCT AAA ACC GTT ACT AAG ACT GTT ATT GGT CCT GAT     1446
Arg Arg Ser Cys Ser Lys Thr Val Thr Lys Thr Val Ile Gly Pro Asp
            460                 465                 470

GGT CAC AAA GAA GTT ACC AAA GAA GTG GTG ACC TCC GAA GAT GGT TCT     1494
Gly His Lys Glu Val Thr Lys Glu Val Val Thr Ser Glu Asp Gly Ser
        475                 480                 485
```

```
GAC TGT CCC GAG GCA ATG GAT TTA GGC ACA TTG TCT GGC ATA GGT ACT      1542
Asp Cys Pro Glu Ala Met Asp Leu Gly Thr Leu Ser Gly Ile Gly Thr
    490                 495                 500

CTG GAT GGG TTC CGT CAT AGG CAC CCT GAT GAA GCT GCC TTC TTC GAC      1590
Leu Asp Gly Phe Arg His Arg His Pro Asp Glu Ala Ala Phe Phe Asp
505                 510                 515                 520

ACT GCC TCA ACT GGA AAA ACA TTC CCA GGT TTC TTC TCA CCT ATG TTA      1638
Thr Ala Ser Thr Gly Lys Thr Phe Pro Gly Phe Phe Ser Pro Met Leu
                525                 530                 535

GGA GAG TTT GTC AGT GAG ACT GAG TCT AGG GGC TCA GAA TCT GGC ATC      1686
Gly Glu Phe Val Ser Glu Thr Glu Ser Arg Gly Ser Glu Ser Gly Ile
            540                 545                 550

TTC ACA AAT ACA AAG GAA TCC AGT TCT CAT CAC CCT GGG ATA GCT GAA      1734
Phe Thr Asn Thr Lys Glu Ser Ser Ser His His Pro Gly Ile Ala Glu
        555                 560                 565

TTC CCT TCC CGT GGT AAA TCT TCA AGT TAC AGC AAA CAA TTT ACT AGT      1782
Phe Pro Ser Arg Gly Lys Ser Ser Ser Tyr Ser Lys Gln Phe Thr Ser
    570                 575                 580

AGC ACG AGT TAC AAC AGA GGA GAC TCC ACA TTT GAA AGC AAG AGC TAT      1830
Ser Thr Ser Tyr Asn Arg Gly Asp Ser Thr Phe Glu Ser Lys Ser Tyr
585                 590                 595                 600

AAA ATG GCA GAT GAG GCC GGA AGT GAA GCC GAT CAT GAA GGA ACA CAT      1878
Lys Met Ala Asp Glu Ala Gly Ser Glu Ala Asp His Glu Gly Thr His
                605                 610                 615

AGC ACC AAG AGA GGG CAT GCT AAA TCT CGC CCT GTC AGA GGT ATC CAC      1926
Ser Thr Lys Arg Gly His Ala Lys Ser Arg Pro Val Arg Gly Ile His
            620                 625                 630

ACT TCT CCT TTG GGG AAG CCT TCC CTG TCC CCC TAGACTAAGT TAAATATTTC    1979
Thr Ser Pro Leu Gly Lys Pro Ser Leu Ser Pro
        635                 640

TGCACAGTGT TCCCATGGCC CCTTGCATTT CCTTCTTAAC TCTCTGTTAC ACGTCATTGA    2039

AACTACACTT TTTTGGTCTG TTTTTGTGCT AGACTGTAAG TTCCTTGGGG GCAGGGCCTT    2099

TGTCTGTCTC ATCTCTGTAT TCCCAAATGC CTAACAGTAC AGAGCCATGA CTCAATAAAT    2159

ACATGTTAAA TGGATGAATG                                                2179
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 643 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Phe Ser Met Arg Ile Val Cys Leu Val Leu Ser Val Val Gly Thr
1 5 10 15

Ala Trp Thr Ala Asp Ser Gly Glu Gly Asp Phe Leu Ala Glu Gly Gly
20 25 30

Gly Val Arg Gly Pro Arg Val Val Glu Arg His Gln Ser Ala Cys Lys
35 40 45

Asp Ser Asp Trp Pro Phe Cys Ser Asp Glu Asp Trp Asn Tyr Lys Cys
50 55 60

Pro Ser Gly Cys Arg Met Lys Gly Leu Ile Asp Glu Val Asn Gln Asp
65 70 75 80

Phe Thr Asn Arg Ile Asn Lys Leu Lys Asn Ser Leu Phe Glu Tyr Gln
85 90 95

Lys Asn Asn Lys Asp Ser His Ser Leu Thr Thr Asn Ile Met Glu Ile
100 105 110

Leu Arg Gly Asp Phe Ser Ser Ala Asn Asn Arg Asp Asn Thr Tyr Asn
115 120 125

Arg Val Ser Glu Asp Leu Arg Ser Arg Ile Glu Val Leu Lys Arg Lys
130 135 140

Val Ile Glu Lys Val Gln His Ile Gln Leu Leu Gln Lys Asn Val Arg
145 150 155 160

Ala Gln Leu Val Asp Met Lys Arg Leu Glu Val Asp Ile Asp Ile Lys
165 170 175

Ile Arg Ser Cys Arg Gly Ser Trp Ser Arg Ala Leu Ala Arg Glu Val
180 185 190

Asp Leu Lys Asp Tyr Glu Asp Gln Gln Lys Gln Leu Glu Gln Val Ile
195 200 205

Ala Lys Asp Leu Leu Pro Ser Arg Asp Arg Gln His Leu Pro Leu Ile
210 215 220

Lys Met Lys Pro Val Pro Asp Leu Val Pro Gly Asn Phe Lys Ser Gln
225 230 235 240

Leu Gln Lys Val Pro Pro Glu Trp Lys Ala Leu Thr Asp Met Pro Gln
245 250 255

Met Arg Met Glu Leu Glu Arg Pro Gly Gly Asn Glu Ile Thr Arg Gly
260 265 270

Gly Ser Thr Ser Tyr Gly Thr Gly Ser Glu Thr Glu Ser Pro Arg Asn
275 280 285

Pro Ser Ser Ala Gly Ser Trp Asn Ser Gly Ser Ser Gly Pro Gly Ser
290 295 300

Thr Gly Asn Arg Asn Pro Gly Ser Ser Gly Thr Gly Gly Thr Ala Thr
305 310 315 320

Trp Lys Pro Gly Ser Ser Gly Pro Gly Ser Ala Gly Ser Trp Asn Ser
325 330 335

Gly Ser Ser Gly Thr Gly Ser Thr Gly Asn Gln Asn Pro Gly Ser Pro
340 345 350

Arg Pro Gly Ser Thr Gly Thr Trp Asn Pro Gly Ser Ser Glu Arg Gly
355 360 365

Ser Ala Gly His Trp Thr Ser Glu Ser Ser Val Ser Gly Ser Thr Gly
370 375 380

Gln Trp His Ser Glu Ser Gly Ser Phe Arg Pro Asp Ser Pro Gly Ser
385 390 395 400

Gly Asn Ala Arg Pro Asn Asn Pro Asp Trp Gly Thr Phe Glu Glu Val
405 410 415

Ser Gly Asn Val Ser Pro Gly Thr Arg Arg Glu Tyr His Thr Glu Lys
420 425 430

Leu Val Thr Lys Gly Asp Lys Glu Leu Arg Thr Gly Lys Glu Lys Val
435 440 445

Thr Ser Gly Ser Thr Thr Thr Thr Arg Arg Ser Cys Ser Lys Thr Val
450 455 460

Thr Lys Thr Val Ile Gly Pro Asp Gly His Lys Glu Val Thr Lys Glu
465 470 475 480

Val Val Thr Ser Glu Asp Gly Ser Asp Cys Pro Glu Ala Met Asp Leu
485 490 495

Gly Thr Leu Ser Gly Ile Gly Thr Leu Asp Gly Phe Arg His Arg His
500 505 510

Pro Asp Glu Ala Ala Phe Phe Asp Thr Ala Ser Thr Gly Lys Thr Phe
515 520 525

```
Pro Gly Phe Phe Ser Pro Met Leu Gly Glu Phe Val Ser Glu Thr Glu
    530                 535                 540

Ser Arg Gly Ser Glu Ser Gly Ile Phe Thr Asn Thr Lys Glu Ser Ser
545                 550                 555                 560

Ser His His Pro Gly Ile Ala Glu Phe Pro Ser Arg Gly Lys Ser Ser
                565                 570                 575

Ser Tyr Ser Lys Gln Phe Thr Ser Ser Thr Ser Tyr Asn Arg Gly Asp
            580                 585                 590

Ser Thr Phe Glu Ser Lys Ser Tyr Lys Met Ala Asp Glu Ala Gly Ser
        595                 600                 605

Glu Ala Asp His Glu Gly Thr His Ser Thr Lys Arg Gly His Ala Lys
    610                 615                 620

Ser Arg Pro Val Arg Gly Ile His Thr Ser Pro Leu Gly Lys Pro Ser
625                 630                 635                 640

Leu Ser Pro
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4027 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..4013

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
AC ATG GCA GTG AGT CAT GGG AGG GAG AGC AAG CCT CTG ACT GCT CAA      47
   Met Ala Val Ser His Gly Arg Glu Ser Lys Pro Leu Thr Ala Gln
    1               5                  10                  15

CAG ACA ACC AAA CTG GAT GCT CCC ACT AAC CTC CAG TTT GTC AAT GAA      95
Gln Thr Thr Lys Leu Asp Ala Pro Thr Asn Leu Gln Phe Val Asn Glu
                20                  25                  30

ACT GAT TCT ACT GTC CTG GTG AGA TGG ACT CCA CCT CGG GCC CAG ATA     143
Thr Asp Ser Thr Val Leu Val Arg Trp Thr Pro Pro Arg Ala Gln Ile
            35                  40                  45
```

```
ACA GGA TAC CGA CTG ACC GTG GGC CTT ACC CGA AGA GGC CAG CCC AGG        191
Thr Gly Tyr Arg Leu Thr Val Gly Leu Thr Arg Arg Gly Gln Pro Arg
          50                  55                  60

CAG TAC AAT GTG GGT CCC TCT GTC TCC AAG TAC CCC CTG AGG AAT CTG        239
Gln Tyr Asn Val Gly Pro Ser Val Ser Lys Tyr Pro Leu Arg Asn Leu
      65                  70                  75

CAG CCT GCA TCT GAG TAC ACC GTA TCC CTC GTG GCC ATA AAG GGC AAC        287
Gln Pro Ala Ser Glu Tyr Thr Val Ser Leu Val Ala Ile Lys Gly Asn
 80                  85                  90                  95

CAA GAG AGC CCC AAA GCC ACT GGA GTC TTT ACC ACA CTG CAG CCT GGG        335
Gln Glu Ser Pro Lys Ala Thr Gly Val Phe Thr Thr Leu Gln Pro Gly
                100                 105                 110

AGC TCT ATT CCA CCT TAC AAC ACC GAG GTG ACT GAG ACC ACC ATC GTG        383
Ser Ser Ile Pro Pro Tyr Asn Thr Glu Val Thr Glu Thr Thr Ile Val
            115                 120                 125

ATC ACA TGG ACG CCT GCT CCA AGA ATT GGT TTT AAG CTG GGT GTA CGA        431
Ile Thr Trp Thr Pro Ala Pro Arg Ile Gly Phe Lys Leu Gly Val Arg
        130                 135                 140

CCA AGC CAG GGA GGA GAG GCA CCA CGA GAA GTG ACT TCA GAC TCA GGA        479
Pro Ser Gln Gly Gly Glu Ala Pro Arg Glu Val Thr Ser Asp Ser Gly
    145                 150                 155

AGC ATC GTT GTG TCC GGC TTG ACT CCA GGA GTA GAA TAC GTC TAC ACC        527
Ser Ile Val Val Ser Gly Leu Thr Pro Gly Val Glu Tyr Val Tyr Thr
160                 165                 170                 175

ATC CAA GTC CTG AGA GAT GGA CAG GAA AGA GAT GCG CCA ATT GTA AAC        575
Ile Gln Val Leu Arg Asp Gly Gln Glu Arg Asp Ala Pro Ile Val Asn
                180                 185                 190

AAA GTG GTG ACA CCA TTG TCT CCA CCA ACA AAC TTG CAT CTG GAG GCA        623
Lys Val Val Thr Pro Leu Ser Pro Pro Thr Asn Leu His Leu Glu Ala
            195                 200                 205

AAC CCT GAC ACT GGA GTG CTC ACA GTC TCC TGG GAG AGG AGC ACC ACC        671
Asn Pro Asp Thr Gly Val Leu Thr Val Ser Trp Glu Arg Ser Thr Thr
        210                 215                 220

CCA GAC ATT ACT GGT TAT AGA ATT ACC ACA ACC CCT ACA AAC GGC CAG        719
Pro Asp Ile Thr Gly Tyr Arg Ile Thr Thr Thr Pro Thr Asn Gly Gln
    225                 230                 235

CAG GGA AAT TCT TTG GAA GAA GTG GTC CAT GCT GAT CAG AGC TCC TGC        767
Gln Gly Asn Ser Leu Glu Glu Val Val His Ala Asp Gln Ser Ser Cys
240                 245                 250                 255

ACT TTT GAT AAC CTG AGT CCC GGC CTG GAG TAC AAT GTC AGT GTT TAC        815
Thr Phe Asp Asn Leu Ser Pro Gly Leu Glu Tyr Asn Val Ser Val Tyr
                260                 265                 270
```

```
ACT GTC AAG GAT GAC AAG GAA AGT GTC CCT ATC TCT GAT ACC ATC ATC        863
Thr Val Lys Asp Asp Lys Glu Ser Val Pro Ile Ser Asp Thr Ile Ile
            275                 280                 285

CCA GAG GTG CCC CAA CTC ACT GAC CTA AGC TTT GTT GAT ATA ACC GAT        911
Pro Glu Val Pro Gln Leu Thr Asp Leu Ser Phe Val Asp Ile Thr Asp
        290                 295                 300

TCA AGC ATC GGC CTG AGG TGG ACC CCG CTA AAC TCT TCC ACC ATT ATT        959
Ser Ser Ile Gly Leu Arg Trp Thr Pro Leu Asn Ser Ser Thr Ile Ile
    305                 310                 315

GGG TAC CGC ATC ACA GTA GTT GCG GCA GGA GAA GGT ATC CCT ATT TTT       1007
Gly Tyr Arg Ile Thr Val Val Ala Ala Gly Glu Gly Ile Pro Ile Phe
320                 325                 330                 335

GAA GAT TTT GTG TAC TCC TCA GTA GGA TAC TAC ACA GTC ACA GGG CTG       1055
Glu Asp Phe Val Tyr Ser Ser Val Gly Tyr Tyr Thr Val Thr Gly Leu
                340                 345                 350

GAG CCG GGC ATT GAC TAT GAT ATC AGC GTT ATC ACT CTC ATT AAT GGC       1103
Glu Pro Gly Ile Asp Tyr Asp Ile Ser Val Ile Thr Leu Ile Asn Gly
            355                 360                 365

GGC GAG AGT GCC CCT ACT ACA CTG ACA CAA CAA ACG GCT GTT CCT CCT       1151
Gly Glu Ser Ala Pro Thr Thr Leu Thr Gln Gln Thr Ala Val Pro Pro
        370                 375                 380

CCC ACT GAC CTG CGA TTC ACC AAC ATT GGT CCA GAC ACC ATG CGT GTC       1199
Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg Val
    385                 390                 395

ACC TGG GCT CCA CCC CCA TCC ATT GAT TTA ACC AAC TTC CTG GTG CGT       1247
Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu Val Arg
400                 405                 410                 415

TAC TCA CCT GTG AAA AAT GAG GAA GAT GTT GCA GAG TTG TCA ATT TCT       1295
Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu Ser Ile Ser
                420                 425                 430

CCT TCA GAC AAT GCA GTG GTC TTA ACA AAT CTC CTG CCT GGT ACA GAA       1343
Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu Pro Gly Thr Glu
            435                 440                 445

TAT GTA GTG AGT GTC TCC AGT GTC TAC GAA CAA CAT GAG AGC ACA CCT       1391
Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln His Glu Ser Thr Pro
        450                 455                 460

CTT AGA GGA AGA CAG AAA ACA GGT CTT GAT TCC CCA ACT GGC ATT GAC       1439
Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp Ser Pro Thr Gly Ile Asp
    465                 470                 475
```

```
TTT TCT GAT ATT ACT GCC AAC TCT TTT ACT GTG CAC TGG ATT GCT CCT      1487
Phe Ser Asp Ile Thr Ala Asn Ser Phe Thr Val His Trp Ile Ala Pro
480                 485                 490                 495

CGA GCC ACC ATC ACT GGC TAC AGG ATC CGC CAT CAT CCC GAG CAC TTC      1535
Arg Ala Thr Ile Thr Gly Tyr Arg Ile Arg His His Pro Glu His Phe
                500                 505                 510

AGT GGG AGA CCT CGA GAA GAT CGG GTG CCC CAC TCT CGG AAT TCC ATC      1583
Ser Gly Arg Pro Arg Glu Asp Arg Val Pro His Ser Arg Asn Ser Ile
            515                 520                 525

ACC CTC ACC AAC CTC ACT CCA GGC ACA GAG TAT GTG GTC AGC ATC GTT      1631
Thr Leu Thr Asn Leu Thr Pro Gly Thr Glu Tyr Val Val Ser Ile Val
        530                 535                 540

GCT CTT AAT GGC AGA GAG GAA AGT CCC TTA TTG ATT GGC CAA CAA TCA      1679
Ala Leu Asn Gly Arg Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser
    545                 550                 555

ACA GTT TCT GAT GTT CCG AGG GAC CTG GAA GTT GTT GCT GCG ACC CCC      1727
Thr Val Ser Asp Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro
560                 565                 570                 575

ACC AGC CTA CTG ATC AGC TGG GAT GCT CCT GCT GTC ACA GTG AGA TAT      1775
Thr Ser Leu Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr
                580                 585                 590

TAC AGG ATC ACT TAC GGA GAA ACA GGA GGA AAT AGC CCT GTC CAG GAG      1823
Tyr Arg Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu
            595                 600                 605

TTC ACT GTG CCT GGG AGC AAG TCT ACA GCT ACC ATC AGC GGC CTT AAA      1871
Phe Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
        610                 615                 620

CCT GGA GTT GAT TAT ACC ATC ACT GTG TAT GCT GTC ACT GGC CGT GGA      1919
Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg Gly
    625                 630                 635

GAC AGC CCC GCA AGC AGC AAG CCA ATT TCC ATT AAT TAC CGA ACA GAA      1967
Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg Thr Glu
640                 645                 650                 655

ATT GAC AAA CCA TCC CAG ATG CAA GTG ACC GAT GTT CAG GAC AAC AGC      2015
Ile Asp Lys Pro Ser Gln Met Gln Val Thr Asp Val Gln Asp Asn Ser
                660                 665                 670

ATT AGT GTC AAG TGG CTG CCT TCA AGT TCC CCT GTT ACT GGT TAC AGA      2063
Ile Ser Val Lys Trp Leu Pro Ser Ser Ser Pro Val Thr Gly Tyr Arg
            675                 680                 685

GTA ACC ACC ACT CCC AAA AAT GGA CCA GGA CCA ACA AAA ACT AAA ACT      2111
Val Thr Thr Thr Pro Lys Asn Gly Pro Gly Pro Thr Lys Thr Lys Thr
        690                 695                 700
```

```
GCA GGT CCA GAT CAA ACA GAA ATG ACT ATT GAA GGC TTG CAG CCC ACA      2159
Ala Gly Pro Asp Gln Thr Glu Met Thr Ile Glu Gly Leu Gln Pro Thr
    705                 710                 715

GTG GAG TAT GTG GTT AGT GTC TAT GCT CAG AAT CCA AGC GGA GAG AGT      2207
Val Glu Tyr Val Val Ser Val Tyr Ala Gln Asn Pro Ser Gly Glu Ser
720                 725                 730                 735

CAG CCT CTG GTT CAG ACT GCA GTA ACC AAC ATT GAT CGC CCT AAA GGA      2255
Gln Pro Leu Val Gln Thr Ala Val Thr Asn Ile Asp Arg Pro Lys Gly
                740                 745                 750

CTG GCA TTC ACT GAT GTG GAT GTC GAT TCC ATC AAA ATT GCT TGG GAA      2303
Leu Ala Phe Thr Asp Val Asp Val Asp Ser Ile Lys Ile Ala Trp Glu
            755                 760                 765

AGC CCA CAG GGG CAA GTT TCC AGG TAC AGG GTG ACC TAC TCG AGC CCT      2351
Ser Pro Gln Gly Gln Val Ser Arg Tyr Arg Val Thr Tyr Ser Ser Pro
        770                 775                 780

GAG GAT GGA ATC CAT GAG CTA TTC CCT GCA CCT GAT GGT GAA GAA GAC      2399
Glu Asp Gly Ile His Glu Leu Phe Pro Ala Pro Asp Gly Glu Glu Asp
    785                 790                 795

ACT GCA GAG CTG CAA GGC CTC AGA CCG GGT TCT GAG TAC ACA GTC AGT      2447
Thr Ala Glu Leu Gln Gly Leu Arg Pro Gly Ser Glu Tyr Thr Val Ser
800                 805                 810                 815

GTG GTT GCC TTG CAC GAT GAT ATG GAG AGC CAG CCC CTG ATT GGA ACC      2495
Val Val Ala Leu His Asp Asp Met Glu Ser Gln Pro Leu Ile Gly Thr
                820                 825                 830

CAG TCC ACA GCT ATT CCT GCA CCA ACT GAC CTG AAG TTC ACT CAG GTC      2543
Gln Ser Thr Ala Ile Pro Ala Pro Thr Asp Leu Lys Phe Thr Gln Val
            835                 840                 845

ACA CCC ACA AGC CTG AGC GCC CAG TGG ACA CCA CCC AAT GTT CAG CTC      2591
Thr Pro Thr Ser Leu Ser Ala Gln Trp Thr Pro Pro Asn Val Gln Leu
        850                 855                 860

ACT GGA TAT CGA GTG CGG GTG ACC CCC AAG GAG AAG ACC GGA CCA ATG      2639
Thr Gly Tyr Arg Val Arg Val Thr Pro Lys Glu Lys Thr Gly Pro Met
    865                 870                 875

AAA GAA ATC AAC CTT GCT CCT GAC AGC TCA TCC GTG GTT GTA TCA GGA      2687
Lys Glu Ile Asn Leu Ala Pro Asp Ser Ser Ser Val Val Val Ser Gly
880                 885                 890                 895

CTT ATG GTG GCC ACC AAA TAT GAA GTG AGT GTC TAT GCT CTT AAG GAC      2735
Leu Met Val Ala Thr Lys Tyr Glu Val Ser Val Tyr Ala Leu Lys Asp
                900                 905                 910
```

```
ACT TTG ACA AGC AGA CCA GCT CAG GGT GTT GTC ACC ACT CTG GAG GGA      2783
Thr Leu Thr Ser Arg Pro Ala Gln Gly Val Val Thr Thr Leu Glu Gly
            915                 920                 925

GGA AAT TTT AAG AGC CAG CTT CAG AAG GTA CCC CCA GAG TGG AAG GCA      2831
Gly Asn Phe Lys Ser Gln Leu Gln Lys Val Pro Pro Glu Trp Lys Ala
        930                 935                 940

TTA ACA GAC ATG CCG CAG ATG AGA ATG GAG TTA GAG AGA CCT GGT GGA      2879
Leu Thr Asp Met Pro Gln Met Arg Met Glu Leu Glu Arg Pro Gly Gly
    945                 950                 955

AAT GAG ATT ACT CGA GGA GGC TCC ACC TCT TAT GGA ACC GGA TCA GAG      2927
Asn Glu Ile Thr Arg Gly Gly Ser Thr Ser Tyr Gly Thr Gly Ser Glu
960                 965                 970                 975

ACG GAA AGC CCC AGG AAC CCT AGC AGT GCT GGA AGC TGG AAC TCT GGG      2975
Thr Glu Ser Pro Arg Asn Pro Ser Ser Ala Gly Ser Trp Asn Ser Gly
                980                 985                 990

AGC TCT GGA CCT GGA AGT ACT GGA AAC CGA AAC CCT GGG AGC TCT GGG      3023
Ser Ser Gly Pro Gly Ser Thr Gly Asn Arg Asn Pro Gly Ser Ser Gly
            995                 1000                1005

ACT GGA GGG ACT GCA ACC TGG AAA CCT GGG AGC TCT GGA CCT GGA AGT      3071
Thr Gly Gly Thr Ala Thr Trp Lys Pro Gly Ser Ser Gly Pro Gly Ser
        1010                1015                1020

GCT GGA AGC TGG AAC TCT GGG AGC TCT GGA ACT GGA AGT ACT GGA AAC      3119
Ala Gly Ser Trp Asn Ser Gly Ser Ser Gly Thr Gly Ser Thr Gly Asn
    1025                1030                1035

CAA AAC CCT GGG AGC CCT AGA CCT GGT AGT ACC GGA ACC TGG AAT CCT      3167
Gln Asn Pro Gly Ser Pro Arg Pro Gly Ser Thr Gly Thr Trp Asn Pro
1040                1045                1050                1055

GGC AGC TCT GAA CGC GGA AGT GCT GGG CAC TGG ACC TCT GAG AGC TCT      3215
Gly Ser Ser Glu Arg Gly Ser Ala Gly His Trp Thr Ser Glu Ser Ser
                1060                1065                1070

GTA TCT GGT AGT ACT GGA CAA TGG CAC TCT GAA TCT GGA AGT TTT AGG      3263
Val Ser Gly Ser Thr Gly Gln Trp His Ser Glu Ser Gly Ser Phe Arg
            1075                1080                1085

CCA GAT AGC CCA GGC TCT GGG AAC GCG AGG CCT AAC AAC CCA GAC TGG      3311
Pro Asp Ser Pro Gly Ser Gly Asn Ala Arg Pro Asn Asn Pro Asp Trp
        1090                1095                1100

GGC ACA TTT GAA GAG GTG TCA GGA AAT GTA AGT CCA GGG ACA AGG AGA      3359
Gly Thr Phe Glu Glu Val Ser Gly Asn Val Ser Pro Gly Thr Arg Arg
    1105                1110                1115

GAG TAC CAC ACA GAA AAA CTG GTC ACT AAA GGA GAT AAA GAG CTC AGG      3407
Glu Tyr His Thr Glu Lys Leu Val Thr Lys Gly Asp Lys Glu Leu Arg
1120                1125                1130                1135
```

```
ACT GGT AAA GAG AAG GTC ACC TCT GGT AGC ACA ACC ACC ACG CGT CGT      3455
Thr Gly Lys Glu Lys Val Thr Ser Gly Ser Thr Thr Thr Arg Arg
                1140                1145                1150

TCA TGC TCT AAA ACC GTT ACT AAG ACT GTT ATT GGT CCT GAT GGT CAC      3503
Ser Cys Ser Lys Thr Val Thr Lys Thr Val Ile Gly Pro Asp Gly His
            1155                1160                1165

AAA GAA GTT ACC AAA GAA GTG GTG ACC TCC GAA GAT GGT TCT GAC TGT      3551
Lys Glu Val Thr Lys Glu Val Val Thr Ser Glu Asp Gly Ser Asp Cys
        1170                1175                1180

CCC GAG GCA ATG GAT TTA GGC ACA TTG TCT GGC ATA GGT ACT CTG GAT      3599
Pro Glu Ala Met Asp Leu Gly Thr Leu Ser Gly Ile Gly Thr Leu Asp
    1185                1190                1195

GGG TTC CGC CAT AGG CAC CCT GAT GAA GCT GCC TTC TTC GAC ACT GCC      3647
Gly Phe Arg His Arg His Pro Asp Glu Ala Ala Phe Phe Asp Thr Ala
1200                1205                1210                1215

TCA ACT GGA AAA ACA TTC CCA GGT TTC TTC TCA CCT ATG TTA GGA GAG      3695
Ser Thr Gly Lys Thr Phe Pro Gly Phe Phe Ser Pro Met Leu Gly Glu
                1220                1225                1230

TTT GTC AGT GAG ACT GAG TCT AGG GGC TCA GAA TCT GGC ATC TTC ACA      3743
Phe Val Ser Glu Thr Glu Ser Arg Gly Ser Glu Ser Gly Ile Phe Thr
            1235                1240                1245

AAT ACA AAG GAA TCC AGT TCT CAT CAC CCT GGG ATA GCT GAA TTC CCT      3791
Asn Thr Lys Glu Ser Ser Ser His His Pro Gly Ile Ala Glu Phe Pro
        1250                1255                1260

TCC CGT GGT AAA TCT TCA AGT TAC AGC AAA CAA TTT ACT AGT AGC ACG      3839
Ser Arg Gly Lys Ser Ser Ser Tyr Ser Lys Gln Phe Thr Ser Ser Thr
    1265                1270                1275

AGT TAC AAC AGA GGA GAC TCC ACA TTT GAA AGC AAG AGC TAT AAA ATG      3887
Ser Tyr Asn Arg Gly Asp Ser Thr Phe Glu Ser Lys Ser Tyr Lys Met
1280                1285                1290                1295

GCA GAT GAG GCC GGA AGT GAA GCC GAT CAT GAA GGA ACA CAT AGC ACC      3935
Ala Asp Glu Ala Gly Ser Glu Ala Asp His Glu Gly Thr His Ser Thr
                1300                1305                1310

AAG AGA GGC CAT GCT AAA TCT CGC CCT GTC AGA GGT ATC CAC ACT TCT      3983
Lys Arg Gly His Ala Lys Ser Arg Pro Val Arg Gly Ile His Thr Ser
            1315                1320                1325

CCT TTG GGG AAG CCT TCC CTG TCC CCC TAGACTAAGT TAAATAT               4027
Pro Leu Gly Lys Pro Ser Leu Ser Pro
        1330                1335
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1336 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Ala Val Ser His Gly Arg Glu Ser Lys Pro Leu Thr Ala Gln Gln
 1               5                  10                  15

Thr Thr Lys Leu Asp Ala Pro Thr Asn Leu Gln Phe Val Asn Glu Thr
            20                  25                  30

Asp Ser Thr Val Leu Val Arg Trp Thr Pro Pro Arg Ala Gln Ile Thr
        35                  40                  45

Gly Tyr Arg Leu Thr Val Gly Leu Thr Arg Arg Gly Gln Pro Arg Gln
    50                  55                  60

Tyr Asn Val Gly Pro Ser Val Ser Lys Tyr Pro Leu Arg Asn Leu Gln
65                  70                  75                  80

Pro Ala Ser Glu Tyr Thr Val Ser Leu Val Ala Ile Lys Gly Asn Gln
                85                  90                  95

Glu Ser Pro Lys Ala Thr Gly Val Phe Thr Thr Leu Gln Pro Gly Ser
            100                 105                 110

Ser Ile Pro Pro Tyr Asn Thr Glu Val Thr Glu Thr Thr Ile Val Ile
        115                 120                 125

Thr Trp Thr Pro Ala Pro Arg Ile Gly Phe Lys Leu Gly Val Arg Pro
    130                 135                 140

Ser Gln Gly Gly Glu Ala Pro Arg Glu Val Thr Ser Asp Ser Gly Ser
145                 150                 155                 160

Ile Val Val Ser Gly Leu Thr Pro Gly Val Glu Tyr Val Tyr Thr Ile
                165                 170                 175

Gln Val Leu Arg Asp Gly Gln Glu Arg Asp Ala Pro Ile Val Asn Lys
            180                 185                 190

Val Val Thr Pro Leu Ser Pro Pro Thr Asn Leu His Leu Glu Ala Asn
        195                 200                 205

Pro Asp Thr Gly Val Leu Thr Val Ser Trp Glu Arg Ser Thr Thr Pro
    210                 215                 220

Asp Ile Thr Gly Tyr Arg Ile Thr Thr Thr Pro Thr Asn Gly Gln Gln
225                 230                 235                 240
```

Gly Asn Ser Leu Glu Glu Val Val His Ala Asp Gln Ser Ser Cys Thr
245 250 255

Phe Asp Asn Leu Ser Pro Gly Leu Glu Tyr Asn Val Ser Val Tyr Thr
260 265 270

Val Lys Asp Asp Lys Glu Ser Val Pro Ile Ser Asp Thr Ile Ile Pro
275 280 285

Glu Val Pro Gln Leu Thr Asp Leu Ser Phe Val Asp Ile Thr Asp Ser
290 295 300

Ser Ile Gly Leu Arg Trp Thr Pro Leu Asn Ser Ser Thr Ile Ile Gly
305 310 315 320

Tyr Arg Ile Thr Val Val Ala Ala Gly Glu Gly Ile Pro Ile Phe Glu
325 330 335

Asp Phe Val Tyr Ser Ser Val Gly Tyr Tyr Thr Val Thr Gly Leu Glu
340 345 350

Pro Gly Ile Asp Tyr Asp Ile Ser Val Ile Thr Leu Ile Asn Gly Gly
355 360 365

Glu Ser Ala Pro Thr Thr Leu Thr Gln Gln Thr Ala Val Pro Pro Pro
370 375 380

Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg Val Thr
385 390 395 400

Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu Val Arg Tyr
405 410 415

Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu Ser Ile Ser Pro
420 425 430

Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu Pro Gly Thr Glu Tyr
435 440 445

Val Val Ser Val Ser Ser Val Tyr Glu Gln His Glu Ser Thr Pro Leu
450 455 460

Arg Gly Arg Gln Lys Thr Gly Leu Asp Ser Pro Thr Gly Ile Asp Phe
465 470 475 480

Ser Asp Ile Thr Ala Asn Ser Phe Thr Val His Trp Ile Ala Pro Arg
485 490 495

Ala Thr Ile Thr Gly Tyr Arg Ile Arg His His Pro Glu His Phe Ser
500 505 510

Gly Arg Pro Arg Glu Asp Arg Val Pro His Ser Arg Asn Ser Ile Thr
515 520 525

Leu Thr Asn Leu Thr Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala
530 535 540

Leu Asn Gly Arg Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr
545 550 555 560

Val Ser Asp Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr
565 570 575

Ser Leu Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr
580 585 590

Arg Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
595 600 605

Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys Pro
610 615 620

Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg Gly Asp
625 630 635 640

Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg Thr Glu Ile
645 650 655

Asp Lys Pro Ser Gln Met Gln Val Thr Asp Val Gln Asp Asn Ser Ile
660 665 670

Ser Val Lys Trp Leu Pro Ser Ser Ser Pro Val Thr Gly Tyr Arg Val
675 680 685

Thr Thr Thr Pro Lys Asn Gly Pro Gly Pro Thr Lys Thr Lys Thr Ala
690 695 700

Gly Pro Asp Gln Thr Glu Met Thr Ile Glu Gly Leu Gln Pro Thr Val
705 710 715 720

Glu Tyr Val Val Ser Val Tyr Ala Gln Asn Pro Ser Gly Glu Ser Gln
725 730 735

Pro Leu Val Gln Thr Ala Val Thr Asn Ile Asp Arg Pro Lys Gly Leu
740 745 750

Ala Phe Thr Asp Val Asp Val Asp Ser Ile Lys Ile Ala Trp Glu Ser
755 760 765

Pro Gln Gly Gln Val Ser Arg Tyr Arg Val Thr Tyr Ser Ser Pro Glu
770 775 780

Asp Gly Ile His Glu Leu Phe Pro Ala Pro Asp Gly Glu Glu Asp Thr
785 790 795 800

Ala Glu Leu Gln Gly Leu Arg Pro Gly Ser Glu Tyr Thr Val Ser Val
805 810 815

Val Ala Leu His Asp Asp Met Glu Ser Gln Pro Leu Ile Gly Thr Gln
820 825 830

Ser Thr Ala Ile Pro Ala Pro Thr Asp Leu Lys Phe Thr Gln Val Thr
835 840 845

Pro Thr Ser Leu Ser Ala Gln Trp Thr Pro Pro Asn Val Gln Leu Thr
850 855 860

Gly Tyr Arg Val Arg Val Thr Pro Lys Glu Lys Thr Gly Pro Met Lys
865 870 875 880

Glu Ile Asn Leu Ala Pro Asp Ser Ser Ser Val Val Val Ser Gly Leu
885 890 895

Met Val Ala Thr Lys Tyr Glu Val Ser Val Tyr Ala Leu Lys Asp Thr
900 905 910

Leu Thr Ser Arg Pro Ala Gln Gly Val Val Thr Thr Leu Glu Gly Gly
915 920 925

Asn Phe Lys Ser Gln Leu Gln Lys Val Pro Pro Glu Trp Lys Ala Leu
930 935 940

Thr Asp Met Pro Gln Met Arg Met Glu Leu Glu Arg Pro Gly Gly Asn
945 950 955 960

Glu Ile Thr Arg Gly Gly Ser Thr Ser Tyr Gly Thr Gly Ser Glu Thr
965 970 975

Glu Ser Pro Arg Asn Pro Ser Ser Ala Gly Ser Trp Asn Ser Gly Ser
980 985 990

Ser Gly Pro Gly Ser Thr Gly Asn Arg Asn Pro Gly Ser Ser Gly Thr
995 1000 1005

Gly Gly Thr Ala Thr Trp Lys Pro Gly Ser Ser Gly Pro Gly Ser Ala
1010 1015 1020

Gly Ser Trp Asn Ser Gly Ser Ser Gly Thr Gly Ser Thr Gly Asn Gln
1025 1030 1035 1040

Asn Pro Gly Ser Pro Arg Pro Gly Ser Thr Gly Thr Trp Asn Pro Gly
1045 1050 1055

Ser Ser Glu Arg Gly Ser Ala Gly His Trp Thr Ser Glu Ser Ser Val
1060 1065 1070

Ser Gly Ser Thr Gly Gln Trp His Ser Glu Ser Gly Ser Phe Arg Pro
1075 1080 1085

Asp Ser Pro Gly Ser Gly Asn Ala Arg Pro Asn Asn Pro Asp Trp Gly
1090 1095 1100

Thr Phe Glu Glu Val Ser Gly Asn Val Ser Pro Gly Thr Arg Arg Glu
1105 1110 1115 1120

Tyr His Thr Glu Lys Leu Val Thr Lys Gly Asp Lys Glu Leu Arg Thr
1125 1130 1135

Gly Lys Glu Lys Val Thr Ser Gly Ser Thr Thr Thr Thr Arg Arg Ser
1140 1145 1150

Cys Ser Lys Thr Val Thr Lys Thr Val Ile Gly Pro Asp Gly His Lys
1155 1160 1165

Glu Val Thr Lys Glu Val Val Thr Ser Glu Asp Gly Ser Asp Cys Pro
1170 1175 1180

Glu Ala Met Asp Leu Gly Thr Leu Ser Gly Ile Gly Thr Leu Asp Gly
1185 1190 1195 1200

Phe Arg His Arg His Pro Asp Glu Ala Ala Phe Phe Asp Thr Ala Ser
1205 1210 1215

Thr Gly Lys Thr Phe Pro Gly Phe Phe Ser Pro Met Leu Gly Glu Phe
1220 1225 1230

Val Ser Glu Thr Glu Ser Arg Gly Ser Glu Ser Gly Ile Phe Thr Asn
1235 1240 1245

Thr Lys Glu Ser Ser Ser His His Pro Gly Ile Ala Glu Phe Pro Ser
1250 1255 1260

Arg Gly Lys Ser Ser Ser Tyr Ser Lys Gln Phe Thr Ser Ser Thr Ser
1265 1270 1275 1280

Tyr Asn Arg Gly Asp Ser Thr Phe Glu Ser Lys Ser Tyr Lys Met Ala
1285 1290 1295

Asp Glu Ala Gly Ser Glu Ala Asp His Glu Gly Thr His Ser Thr Lys
1300 1305 1310

Arg Gly His Ala Lys Ser Arg Pro Val Arg Gly Ile His Thr Ser Pro
1315 1320 1325

Leu Gly Lys Pro Ser Leu Ser Pro
1330 1335

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC1551

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GATCCCCGGG GAGCTCCTCG AGGCATG 27

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC1552

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCTCGAGGAG CTCCCCGGG 19

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC2052

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AATTCACCAT GGCAGTGAGT 20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC2053

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CATGACTCAC TGCCATGGTG 20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC2491

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTAGATTAGA ATGGGGCC 18

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC2493

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCATTCTAAT 10

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 88 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC3521

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TCGACTTAAG GACACTTTGA CAAGCAGACC AGCTCAGGGT GTTGTCACCA CTCTGGAGGG 60

AGGAAATTTT AAGAGCCAGC TTCAGAAG 88

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 88 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC3522

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GTACCTTCTG AAGCTGGCTC TTAAAATTTC CTCCCTCCAG AGTGGTGACA ACACCCTGAG 60

CTGGTCTGCT TGTCAAAGTG TCCTTAAG 88

I Claim:

1. A hybrid protein comprising a tissue-binding domain from a first protein covalently linked to a cross-linking domain from a second protein.

2. A hybrid protein according to claim 1 wherein the tissue-binding domain of the first protein is a heparin binding domain of thrombospondin, a heparin binding domain of fibronectin, a collagen binding domain of fibronectin or a cell binding domain of fibronectin.

3. A hybrid protein according to claim 1 wherein the tissue-binding domain of the first protein comprises the amino acid sequence of Sequence ID No. 6 from Alanine, amino acid 2 to Glutamic acid, amino acid number 926.

4. A hybrid protein according to claim 1 wherein the cross-linking domain of the second protein comprises the carboxy-terminal 103 amino acids of loricrin; the ten amino acid repeat beginning with glutamine, amino acid number 496 of involucrin; or the 400 amino-terminal amino acids of the fibrinogen α chain.

5. A hybrid protein according to claim 1 wherein the cross-linking domain of the second protein comprises the amino acid sequence of Sequence ID No. 6 from Glycine, amino acid number 928 to Proline, amino acid number 1336.

6. A hybrid protein according to claim 1 comprising the amino acid sequence of Sequence ID Number 6 from alanine, amino acid number 2 to Proline, amino acid number 1336.

7. An isolated DNA molecule encoding a hybrid protein comprising a first DNA segment encoding a tissue-binding domain from a first protein joined to a second DNA segment encoding a cross-linking domain from a second protein.

8. A DNA molecule according to claim 7 wherein the first DNA segment encodes a heparin binding domain of thrombospondin, a heparin binding domain of fibronectin, a collagen binding domain of fibronectin, a collagen binding domain of fibronectin or a cell binding domain of fibronectin.

9. A DNA molecule according to claim 7 wherein the first DNA segment comprises the nucleotide sequence of Sequence ID No. 5 from nucleotide 3 to nucleotide 2780.

10. A DNA molecule according to claim 7 wherein the first DNA segment encodes the amino acid sequence of Sequence ID No. 6 from methionine, amino acid number 1 to glutamic acid, amino acid number 926.

11. A DNA molecule according to claim 7 wherein the second DNA segment encodes the carboxy-terminal 103 amino acids of loricrin; the ten amino acid repeat beginning with glutamine, amino acid number 496 of involucrin; or the 400 amino-terminal amino acids of the fibrinogen α chain.

12. A DNA molecule according to claim 7 wherein the second DNA segment comprises the nucleotide sequence of Sequence ID No. 5 from nucleotide 2784 to nucleotide 4013.

13. A DNA molecule according to claim 7 wherein the second DNA segment encodes the amino acid sequence of Sequence ID No. 6 from glycine, amino acid number 928 to proline, amino acid number 1336.

14. A DNA molecule according to claim 7 wherein the DNA molecule encodes the amino acid sequence of Sequence ID Number 6 from Methionine, amino acid number 1 to Proline, amino acid number 1336.

15. A DNA molecule according to claim 7 wherein the DNA molecule comprises the nucleotide sequence of Sequence ID Number 5 from nucleotide 3 to nucleotide 4013.

16. A DNA construct comprising a DNA molecule encoding a hybrid protein, wherein said DNA molecule comprises a first DNA segment encoding a tissue-binding domain from a first protein joined to a second DNA segment encoding a cross-linking domain from a second protein, and wherein said DNA molecule is operably linked to other DNA segments required for the expression of the DNA molecule.

17. A DNA construct according to claim 16 wherein the first DNA segment encodes a heparin binding domain of thrombospondin, a heparin binding domain of fibronectin, a collagen binding domain of fibronectin or a cell binding domain of fibronectin.

18. A DNA construct according to claim 16 wherein the first DNA segment comprises the nucleotide sequence of Sequence ID No. 5 from nucleotide 3 to nucleotide 2780.

19. A DNA construct according to claim 16 wherein the first DNA segment encodes the amino acid sequence of Sequence ID No. 6 from methionine, amino acid 1 to Glutamic acid, amino acid number 926.

20. A DNA construct according to claim 16 wherein the second DNA segment encodes the carboxy-terminal 103 amino acids of loricrin; the ten amino acid repeat beginning with glutamine, amino acid number 496 of involucrin; or the 400 amino-terminal amino acids of the fibrinogen α chain.

21. A DNA construct according to claim 16 wherein the second DNA segment comprises the nucleotide sequence of Sequence ID No. 5 from nucleotide 2784 to nucleotide 4013.

22. A DNA construct according to claim 16 wherein the second DNA segment encodes the amino acid sequence of Sequence ID No. 6 from glycine, amino acid number 928 to proline, amino acid number 1336.

23. A DNA construct according to claim 16 wherein the DNA molecule comprises the nucleotide sequence of Sequence ID Number 5 from nucleotide 1 to nucleotide 4013.

24. A DNA construct according to claim 16 wherein the DNA molecule encodes the amino acid sequence of Sequence ID Number 6 from Methionine, amino acid number 1 to Proline, amino acid number 1336.

25. A host cell containing a DNA construct according to claim 16.

26. A method for producing a hybrid protein comprising culturing a host cell according to claim 25 under conditions promoting the expression of the first DNA segment.

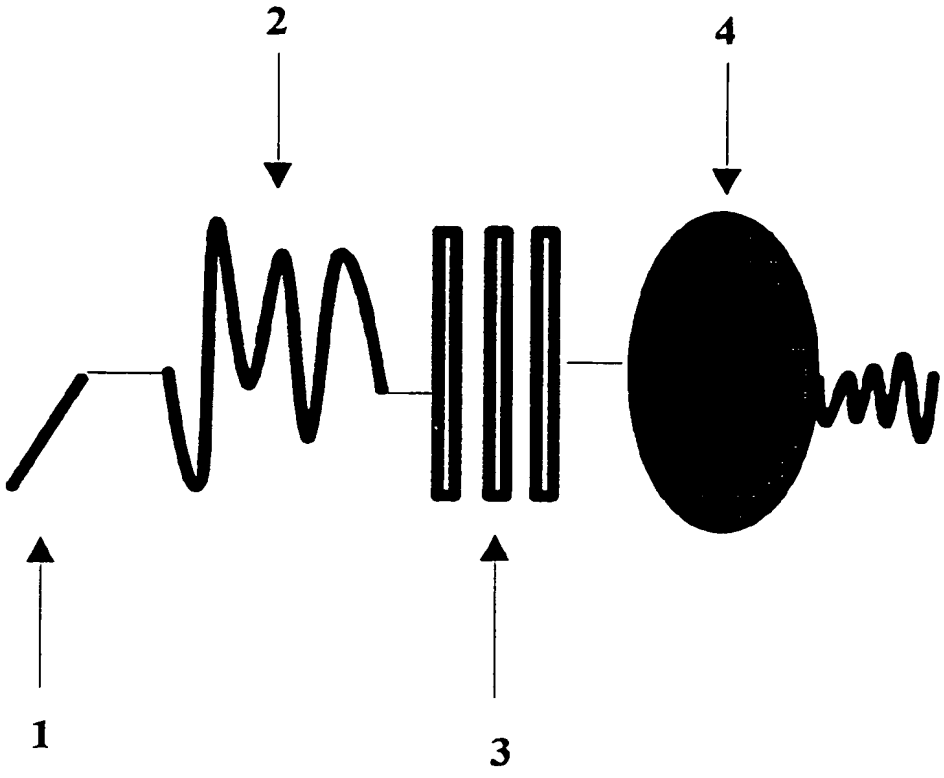


FIGURE 1

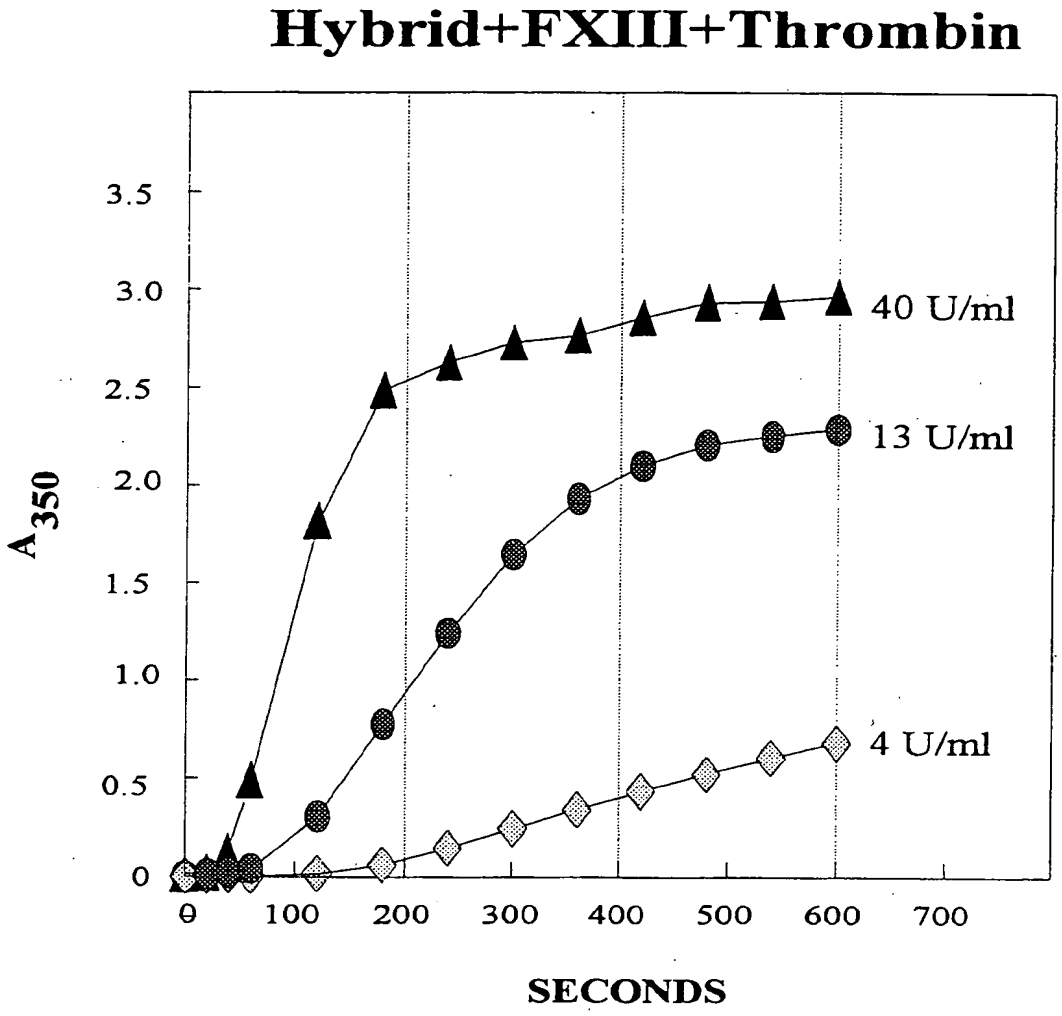

Hybrid+FXIII+Thrombin
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
A350
40 U/ml
13 U/ml
4 U/ml
0
100
200
300
400
500
600
700
SECONDS

FIGURE 2

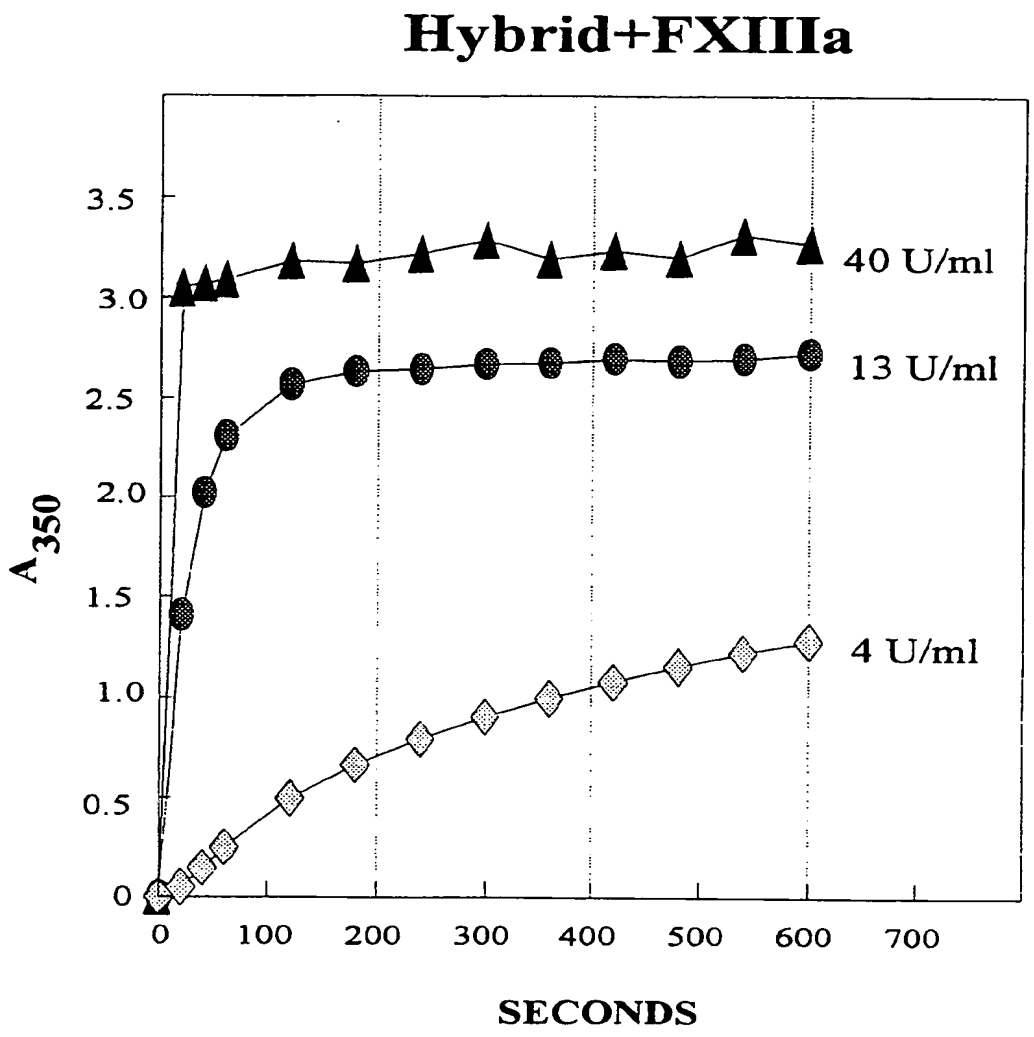

Hybrid+FXIIIa
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
A_{350}
40 U/ml
13 U/ml
4 U/ml
0
100
200
300
400
500
600
700
SECONDS

FIGURE 3

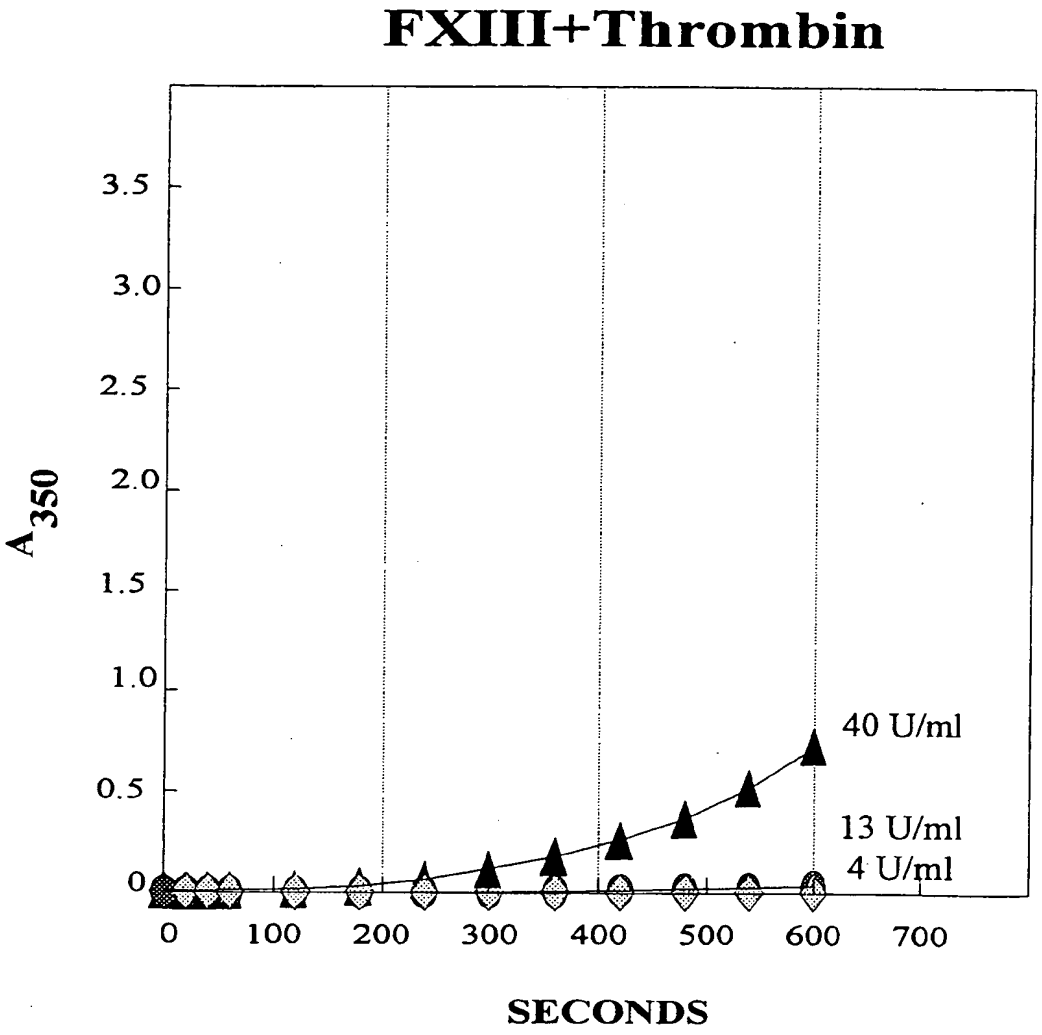

FXIII+Thrombin
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
A_{350}
40 U/ml
13 U/ml
4 U/ml
0
100
200
300
400
500
600
700
SECONDS

FIGURE 4

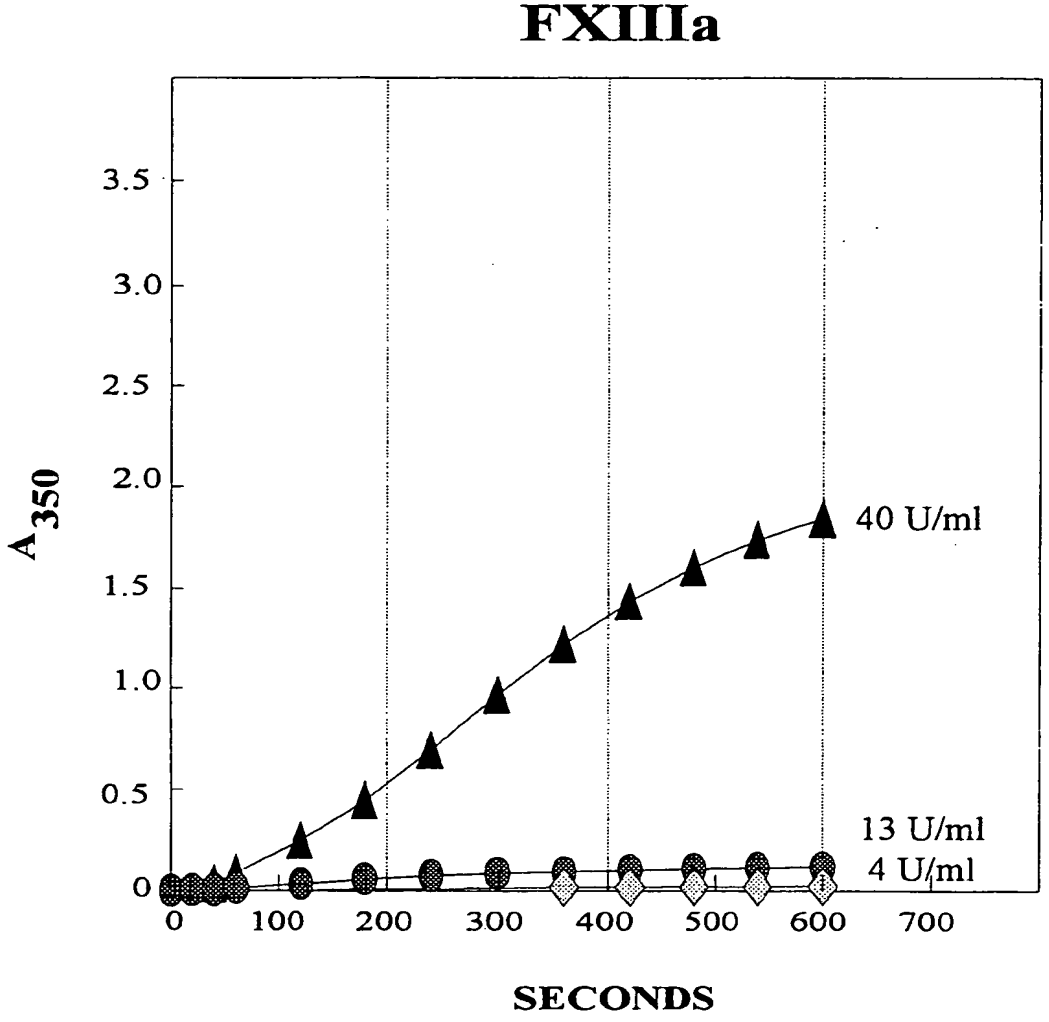

FXIIIa
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
A_{350}
40 U/ml
13 U/ml
4 U/ml
0
100
200
300
400
500
600
700
SECONDS

FIGURE 5